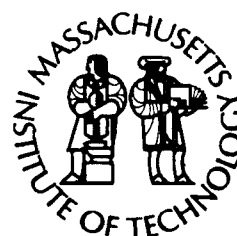# Woods Hole Oceanographic Institution

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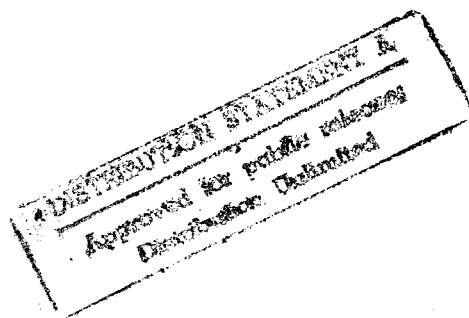### Applied Ocean Science
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Distribution, Abundance and Ecology of Mixotrophic Algae in Marine and Freshwater Plankton Communities

by

Andrea Lynn Arenovski

September 1994

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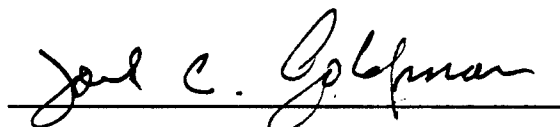Cambridge, Massachusetts 02139

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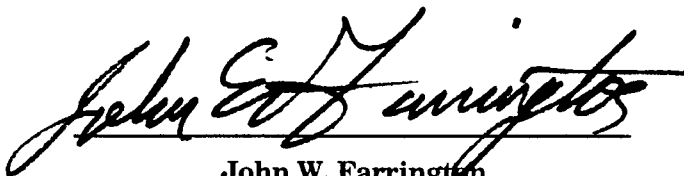
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The Distribution, Abundance and Ecology of Mixotrophic Algae in Marine and Freshwater Plankton Communities

by

Andrea Lynn Arenovski

B.S., University of North Carolina at Wilmington (1988)

Submitted to the Joint Committee for Biological Oceanography
in Partial Fulfillment of the Requirements for
the Degree of Doctor of Philosophy

at the

Massachusetts Institute of Technology

and the

Woods Hole Oceanographic Institution

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Distribution, Abundance and Ecology of Mixotrophic Algae in Marine and
Freshwater Plankton Communities

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Submitted in partial fulfillment of the requirements for the degree of
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ABSTRACT

Mixotrophic algae are algae that combine photosynthesis with phagotrophy to satisfy nutritional requirements. Mixotrophic algae have been found to dominate the nanoplankton assemblage in some aquatic environments, and algal phagotrophy can account for a significant fraction of total bacterivory on occasion. The distribution and abundance of mixotrophic algae have not been widely characterized, however, and the factors that control their abundances and phagotrophic activity in aquatic environments are poorly understood. The vertical distribution and abundance of mixotrophic nanoplankton was therefore documented in a variety of aquatic environments including the oligotrophic open ocean (Sargasso Sea), nine freshwater seepage ponds (Adirondack Mountains, NY) and three coastal salt ponds (Falmouth, MA) using epifluorescence microscopy and fluorescently labeled prey to trace ingestion by algae. Field experiments designed to examine the influence of prey density, nutrient availability and light intensity on mixotrophic nanoplankton abundances and their phagotrophic activity were also carried out to examine the adaptive significance of phagotrophy for mixotrophic algae in surface waters of the Sargasso Sea and in the epilimnion of one freshwater seepage pond.

Mixotrophic nanoplankton abundances in the Sargasso Sea ranged from less than a few cells ml^{-1} to more than 140 cells ml^{-1}, and they were more abundant in surface waters. On two occasions mixotrophic algae comprised more than 50% of the total algal nanoplankton assemblage in surface waters. The results of field experiments indicate that phagotrophy may provide mixotrophic algae in surface waters of the Sargasso Sea with major nutrients and may enable them to compete with purely phototrophic algae during periods of low nutrient concentrations.

Mixotrophic nanoplankton abundances comprised a small fraction of the total algal nanoplankton (<5%) in most of the freshwater seepage ponds and in all three coastal salt ponds. Mixotrophic algae in the epilimnion of one freshwater pond, however, accounted for ~25% of the total algal nanoplankton, and *Dinobryon* dominated the mixotrophic nanoplankton in this pond (~2x10^4 *Dinobryon* ml^{-1}). Results from field experiments support the hypothesis that nutrient acquisition is an important secondary function for phagotrophy in *Dinobryon*, but that the primary function may be to provide essential growth factors.

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CHAPTER 1

Introduction

INTRODUCTION

Over the last two decades we have seen the emergence of a new paradigm of the planktonic food web (Pomeroy, 1974). The new paradigm recognizes a much larger role for minute algae in primary production than was previously thought, and includes an extension of the classical paradigm now known as the microbial food loop. The microbial loop incorporates microheterotrophic processes into the classical food web and is considered important in the cycling of energy and nutrients in planktonic ecosystems. It has become customary to summarize the trophodynamics in the microbial food web by clear distinctions between phototrophy and heterotrophy, and size-dependent predator-prey relationships (Fig. 1.1). Picoplanktonic and nanoplanktonic phototrophs (prokaryotic and eukaryotic algae 0.2-2 μm and 2-20 μm in size, respectively) are now known to dominate the phytoplankton assemblage in many aquatic environments (Ilmavirta, 1982; Davis & Sieburth, 1984; Estep et al., 1984; Davis et al., 1985), and they are believed to account for a significant fraction of primary production (Malone, 1971; McCarthy et al., 1974; Larsson & Hagström, 1982; Hagström et al., 1983; Hannah & Boney, 1983; Joint et al., 1986). It is now believed that as much as 50% of primary production is consumed by picoplanktonic heterotrophs (bacteria 0.2-2 μm in size), and that a large fraction of this production is converted into bacterial biomass rather than remineralized by the bacteria (Sorokin, 1971; Pomeroy, 1974; Sieburth et al., 1977; Larsson & Hagström, 1979; Fuhrman & Azam, 1980; Williams, 1981; Azam et al., 1983). Nanoplankton-sized (2-20 μm) heterotrophic protists (flagellates and ciliates) are believed to be the major consumers of bacterial production (Sieburth, 1979; Sorokin, 1979; Fenchel, 1982c), and are thought to be responsible for much of the remineralization and recycling of essential nutrients for phytoplankton and bacteria (Azam et al., 1983; Caron, 1984; Caron & Goldman, 1990). These nanoplanktonic bacterivores are grazed by larger protists which, in turn, may be consumed by larger

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Size Class (μm)	Phototrophs	Heterotrophs
macroplankton (>200)		sarcodines and metazoa
microplankton (20-200)	pigmented dinoflagellates and diatoms	unpigmented dinoflagellates and ciliates
nanoplankton (2-20)	pigmented flagellates and some prokaryotes	unpigmented flagellates and some ciliates
picoplankton (<2)	chroococcoid cyanobacteria and some eukaryotes	bacteria

Figure 1.1. Summary of microbial food web trophic relationships as defined by size class and nutritional mode.

zooplankton thereby returning some fraction of the energy and nutrients from bacteria to the classical food web via flow through the microbial loop (Sherr & Sherr, 1984; Caron, 1991).

This modern view of microbial trophodynamics has provided a basis for better understanding energy and nutrient flow in planktonic systems. However, shortcomings associated with an approach that makes clear distinctions between phototrophy and heterotrophy have become apparent. In particular, it has become increasingly evident that many protists are capable of functioning at more than one trophic level. The rapid differentiation of phototrophic and heterotrophic plankton based on the presence or absence of plastids (Porter & Feig, 1980; Caron, 1983; Sherr & Sherr, 1983; Sherr *et al.*, 1993) has yielded useful information on the phototrophic abilities of protists, but new methods (Borsheim, 1984; Cynar & Sieburth, 1986; Wikner *et al.*, 1986; Sherr *et al.*, 1987) have resulted in the discovery (or rediscovery) of planktonic protists that are capable of phagotrophy in addition to photosynthesis, and thus, function at more than one trophic level. Mixotrophy refers to the ability of these organisms to combine phototrophy with phagotrophy to satisfy nutritional requirements for growth and/or survival (Sanders, 1991).

The Sarcodinia and Ciliophora are examples of protistan taxa that have generally been considered to be heterotrophic. It is now known that some sarcodines and ciliates contain highly modified endosymbiotic algae that allow them to function primarily as autotrophs, while other species of sarcodines and ciliates are mixotrophic (Laval-Peuto & Brownlee, 1986; Laval-Peuto *et al.*, 1986; Patterson & Dürrschmidt, 1987; Laval-Peuto & Rassoulzadegan, 1988; Stoecker *et al.*, 1988; Rogerson *et al.*, 1989; Stoecker *et al.*, 1989b). These latter mixotrophic species appear to sequester chloroplasts after ingesting algal prey. The chloroplasts, which remain functional once inside the cells, allow the protists to function both phototrophically and phagotrophically (Lopez, 1979; Stoecker *et al.*, 1987; Lee *et al.*, 1988).

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In addition, numerous species of plastidic flagellates from diverse taxa (Chrysophyceae, Prymnesiophyceae, Cryptophyceae and Dinophyceae) have been found to be capable of particle ingestion (for summary of references see Sanders & Porter, 1988). The ability of algae to consume particulate organic material (usually bacteria and other small unicellular organisms) has been known for nearly a century. Until recently though, the ecological implications of this behavior have been largely ignored. Algal phagotrophy has gained renewed interest with recognition of the importance of microheterotrophic processes in planktonic food webs (Williams, 1981; Azam et al., 1983) and methodological developments in the area of microbial ecology that allow direct differentiation of heterotrophic (apochlorotic) protists and phagocytic or nonphagocytic plastidic protists in natural samples.

Some investigations have focused on individual mixotrophic species to determine the role of phagotrophy in their nutrition. These investigations have provided information to suggest that phagotrophy may play a variety of roles, including the acquisition of carbon, major nutrients (nitrogen and phosphorus) or specific growth factors such as vitamins or essential lipids (Kimura & Ishida, 1989; Sanders et al., 1990; Caron et al., 1992; Nygaard & Tobiesen, 1993). Species that are capable of acquiring carbon via phagotrophy may experience a selective advantage over purely phototrophic algae in light limiting conditions, while acquisition of major nutrients (N or P) may be particularly advantageous to algae in oligotrophic environments.

Mixotrophic algae have been found to dominate the nanoplankton assemblage in some aquatic environments, and it is clear from a few field studies that algal phagotrophy can occasionally contribute significantly to total bacterivory (Bird & Kalff, 1986; Porter, 1988; Bird & Kalff, 1989; Sanders et al., 1989; Berninger et al., 1992). Nevertheless, the distribution and abundance of mixotrophic algae have not been widely characterized in aquatic environments, and the factors that control their abundances in these environments are poorly understood. As a consequence, the ecological importance

of mixotrophy in the flow of energy and nutrients through planktonic food webs remains unclear.

The purpose of this dissertation was to further our understanding of the importance of nanoplanktonic mixotrophic algae in aquatic ecosystems and to attempt to determine what factors influence their phagotrophic behavior in these environments. The dissertation is composed of 3 manuscripts which examine the relationships between the distribution and abundance of mixotrophic algae and physical, chemical and biological parameters that may influence their abundance and phagotrophic activity in marine and freshwater plankton communities. The vertical distribution and abundance of mixotrophic nanoplankton at several stations in the Sargasso Sea, south of Bermuda, and the importance of phagotrophy in the nutrition of mixotrophic algae in surface waters is discussed based on the results of experimental manipulations are described in Chapter 2. Information on the density of picoplanktonic and nanoplanktonic phototrophs and heterotrophs throughout the euphotic zone at each station is also provided in this chapter.

Relationships between the abundance of mixotrophic algae and physical, chemical and biological parameters in a series of seepage ponds located in the Adirondack Mountains of New York and in a group of coastal salt ponds located in Falmouth, Massachusetts are examined in Chapter 3. The vertical distributions and abundances of mixotrophic algae are determined and their contribution to the total phototrophic nanoplankton is presented. Densities of heterotrophic bacteria and nanoplankton are also considered in relation to the abundance of mixotrophic algae. In addition, the contribution of mixotrophic ("green") ciliates to ciliate populations in one coastal salt pond are discussed.

Finally, a study conducted in one of the Adirondack seepage ponds examined in the previous chapter is discribed in Chapter 4. Mixotrophic algae were found to be relatively abundant in the epilimnion of this pond. An experiment to determine the

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factors that may control the abundance or phagotrophic activity of mixotrophic algae in this pond are discussed in Chapter 3. Specifically, the influence of light, major nutrients (N and P) and prey density on the phagotrophic activity of an abundant mixotrophic chrysophyte, *Dinobryon* along with other solitary and colonial mixotrophic nanoplankton was examined.

The results from these studies indicate that mixotrophic nanoplankton can be numerically important in some aquatic environments. In addition, field experiments reported on in Chapters 2 and 4 provide the first evidence from the field to suggest that mixotrophic nanoplankton obtain major nutrients (N and P) by consuming bacteria.

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CHAPTER 2

Mixotrophic Nanoplankton in Oligotrophic Surface Waters of the
Sargasso Sea May Employ Phagotrophy to Obtain Major Nutrients

ABSTRACT

The vertical distribution and abundance of mixotrophic nanoplankton was examined in an oligotrophic open ocean plankton community. Fluorescently labeled bacteria and cyanobacteria were used as tracers of ingestion in experiments designed to determine abundances of mixotrophic nanoplankton during two cruises to the Sargasso Sea south of Bermuda. Phagotrophic nanoplanktonic (2-20 μm) algae ranged from undetectable to more than 100 ml^{-1}, and were more abundant near the surface (up to 140 ml^{-1}) than in the deeper euphotic zone. On two occasions 50% of the phototrophic nanoplankton in surface waters were observed with ingested fluorescent tracers. The contribution of mixotrophic algae to the total phototrophic nanoplankton assemblage in the deep chlorophyll maximum, however, did not exceed 0.5%. It is possible that mixotrophic algae were more abundant in the DCM, but were not phagotrophically active.

Two four-day experimental incubations were subsequently carried out to determine the adaptive significance of phagotrophic behavior for algae in surface waters of the Sargasso Sea. Experiments were designed to examine the influence of light, nutrients and prey density on the phagotrophic behavior of mixotrophic nanoplankton. Greater mixotrophic nanoplankton abundances were observed in treatments that received no nutrient inputs and were limited by the availability of inorganic nutrients during the experiments. A decrease in the abundance of mixotrophic algae or a decrease in their phagotrophic activity occurred with nutrient enrichment. Based on the experimental results we suggest that phagotrophy was a mechanism by which these algae supplemented nutrient acquisition during periods of low dissolved nutrient concentrations. Higher abundances of mixotrophic nanoplankton observed in the upper 50 m of the Sargasso Sea may have been due to the generally low nutrient concentrations in these waters.

INTRODUCTION

Mixotrophic algae are algae that combine autotrophic and heterotrophic modes of nutrition. In many microalgae this trophic behavior is characterized by a combination of photosynthesis and the ingestion and digestion of preformed organic material (usually bacteria and other small unicellular microorganisms; Fenchel, 1982b; Sanders & Porter, 1988; Andersson *et al.*, 1989; Caron *et al.*, 1990; Sanders *et al.*, 1990; Sanders, 1991). The ability to ingest particulate material by unicellular algae has been known for many years (Pascher, 1911; Pringsheim, 1952; Pringsheim, 1955). Only recently, though, have ecologists begun to recognize the potential significance of this behavior for algal growth and survival, and elemental flow through aquatic food webs (Bird & Kalff, 1989).

Phagotrophic activity by algae has important implications for algal growth and survival. Phagotrophy may serve as a mechanism for obtaining carbon, major nutrients (nitrogen or phosphorus) or specific growth factors that may be necessary to satisfy the nutritional requirements of the algae. Some facultative mixotrophic species, or amphitrophs (Schnepf & Elbrächter, 1992) are primarily heterotrophic and rely on phagotrophy to obtain most or all of the organic carbon used for metabolism and growth. Photosynthesis in these species appears to be used as a mechanism for survival only under poor heterotrophic conditions (*i.e.*, low prey abundance). For example, phagotrophy was found to be the dominant source of nutrition in the freshwater chrysophyte *Poterioochromonas malhamensis* (Caron *et al.*, 1990; Sanders *et al.*, 1990; Lewitus & Caron, 1991). This alga ingested prey and grew rapidly in a range of light regimes, including continuous darkness if bacteria were present in the culture medium in sufficient numbers. When bacterial abundances were reduced below a grazing threshold of 10^6 bacteria ml^{-1}, however, the alga's growth rate slowed considerably, but continued at a very slow rate provided there was adequate light for photosynthesis. Similar

patterns of grazing, photosynthesis and growth have been observed in some species of *Ochromonas* (Fenchel, 1982a; Andersson *et al.*, 1989).

Other species may be obligate mixotrophs that require both photosynthesis and phagotrophy to sustain growth of the algae. In these species phagotrophy may play a different role. For example, it has been suggested that phagotrophy may be employed by some algae to acquire specific growth factors (*i.e.* vitamins, lipids) required for phototrophic growth. While phycologists have used the term "auxotroph" to refer to algal species with specific organic requirements (Morris, 1980) phagotrophy as a mechanism for obtaining those growth factors has been demonstrated in few instances. Kimura and Ishida (1986) demonstrated that phagotrophic behavior by the mixotrophic freshwater red tide chrysophyte *Uroglena americana* was necessary to sustain growth of the alga. Laboratory studies with *U. americana* revealed that bacteria provided a specific phospholipid that the algae were unable to synthesize (Kimura & Ishida, 1989). Acquisition of a growth factor has also been suggested as one possible explanation for phagotrophic activity in another freshwater chrysophyte, *Dinobryon cylindricum* (Caron *et al.*, 1992).

Phagotrophy may also be used by some mixotrophic algae to acquire major nutrients (nitrogen and phosphorus). There are very few laboratory data to support this hypothesis, however (Veen, 1991; Nygaard & Tobiesen, 1993). Photosynthesis would presumably be the primary energy source for these mixotrophs while phagotrophy would allow these algae to successfully compete with non-phagotrophic algae (and bacteria) for growth-limiting nutrients. Such a strategy for growth might be particularly useful in well-lighted oligotrophic waters.

While the examples given above indicate that phagotrophic behavior may provide mixotrophic algae with a considerable selective advantage over non-mixotrophic species, relatively few data actually exist on the abundance or activity of mixotrophic species in nature. A few studies have demonstrated that mixotrophic algae are abundant

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in freshwater environments and occasionally comprise a large percentage of the phytoplankton community. Bird and Kalff (1989) reported that mixotrophic algae were responsible for sustenance of a metalimnetic phytoplankton peak in Lac Gilbert, Quebec. They determined that phagotrophy might have accounted for nearly 80% of the algal community carbon assimilation in this peak on an overcast day. Berninger et al. (1992) found that over-wintering phytoplankton populations in a Pennsylvanian lake were dominated by mixotrophic (phagotrophic) chrysophyte algae. These species were also common in the phytoplankton bloom that takes place in the lake at the time of ice-out (Siver & Chock, 1986). Berninger et al. (1992) suggested that the inoculum for the bloom may be maintained, in part, by phagotrophy of these algae under the ice.

Several species of mixotrophic algae have been observed and/or cultured from oceanic environments (Fenchel, 1982a; Estep et al., 1986; Andersson et al., 1989; Nygaard & Hessen, 1990; Bockstahler & Coats, 1993) but the overall importance of mixotrophic algae in marine plankton communities remains unclear. The use of phagotrophy to acquire major nutrients has been proposed for some marine species of mixotrophic algae. Over the past several years, toxic blooms of algal flagellates occurred in the coastal waters of Norway. These blooms were associated with low inorganic phosphate concentrations in the water column (Dahl et al., 1989). Interestingly, the blooms were dominated by algal flagellates capable of phagotrophy (Børsheim et al., 1989; Johnsen & Lein, 1989). Nygaard and Tobiesen (1993) examined bacterivory by several species of these marine mixotrophic flagellates in P-limited and non-limited cultures. Based on experiments performed in the laboratory, they hypothesized that phagotrophic behavior may permit these species to form blooms when phosphate becomes limiting to other algae.

The present study was designed to determine the abundance of mixotrophic algae in an oligotrophic open ocean plankton community and to examine experimentally the influence of light, nutrients and prey density on their phagotrophic behavior. Analyses

of water samples collected in the euphotic zone of the Sargasso Sea during a cruise in August 1989 demonstrated the presence of significant but variable numbers of nanoplanktonic mixotrophs. A more detailed examination of the vertical distribution of mixotrophic nanoplankton (MNAN) conducted in early April 1992 yielded a range in abundances of mixotrophs from <1 MNAN ml^{-1} just below the deep chlorophyll maximum to greater than 30 MNAN ml^{-1} near the surface. Experiments conducted to examine the reasons for phagotrophic activity by algae in surface waters during this study support the hypothesis that mixotrophic algae in surface waters of this oligotrophic oceanic system were ingesting bacteria in response to nutrient stress.

METHODS

Mixotrophic nanoplankton abundances during August 1989

Estimates of mixotrophic nanoplankton abundances in euphotic zone waters of the Sargasso Sea were obtained in August 1989 as a part of the ZOOSWAT program (R/V Gyre). These measurements were obtained during experiments designed to estimate the ingestion rates of phagotrophic protists (<20 μm in size) using the FLB uptake method (Sherr et al., 1987). Samples were collected in 0.1 N HCl-rinsed Niskin bottles from two depths at seven closely spaced stations during August 1989 south of Bermuda (28-32°N, 63-78°W). Water samples were collected from a depth between 1 and 5 m (surface) and from a depth coincident with the deep chlorophyll maximum (DCM) as determined by *in situ* fluorometry using a CTD-fluorescence instrument package attached to a Niskin rosette sampler. Subsamples were removed and preserved immediately with gluteraldehyde at a final concentration of 1% for microscopic determination of bacteria and chroococcoid cyanobacteria abundances (<2 μm), and phototrophic and heterotrophic nanoplankton abundances (microalgae and protozoa 2-20 μm in size, respectively). All population counts were performed by epifluorescence microscopy at 1000x magnification (Porter & Feig, 1980; Caron, 1983; Waterbury et al., 1987; Sherr et al., 1993).

Grazing experiments were conducted using fluorescently labeled cultured microorganisms as tracers of natural prey that were prepared according to methods outlined in Sherr et al. (1987). Grazing experiments were conducted using fluorescently labeled cultured microorganisms as tracers of natural prey (Sherr et al., 1987). Fluorescently labeled cyanobacteria (*Synechococcus* sp., clone WH8012) were used extensively in these experiments. This cyanobacterium maintains a small size (~0.8-0.9 μm in diameter) when grown in laboratory cultures and thus is representative of the size

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of naturally occurring cyanobacteria and some heterotrophic bacteria. Fluorescently labeled minicells (FLM) of the bacterium *Escherichia coli* were also used (Pace *et al.*, 1990), but the usefulness of FLM for examining protistan ingestion was compromised by clumping and fading of these cells.

One liter subsamples from each depth were placed in 1.2 L clear polycarbonate vessels and inoculated with fluorescently labeled prey within one hour of collection. Immediately after the addition of fluorescently labeled prey the containers were gently swirled to distribute the tracer, and samples were removed and preserved to establish the initial numbers of labeled prey and to provide a "time zero" sample for examining ingestion. The abundances of labeled prey used in these experiments were chosen such that they could be readily counted, but did not dramatically increase the abundance of natural prey of the same size (generally 10-30% of the natural prey abundance). Incubations were conducted in duplicate in an on-deck Plexiglas incubator. Seawater was continuously pumped through the incubator to maintain the water temperature at ambient surface water temperature. Light intensity was attenuated to the approximate intensities at the two sampling depths using neutral density screening. After 24 hours of incubation a second set of samples was removed and preserved to establish the abundance of mixotrophic nanoplankton.

More than 400 individual phototrophic nanoplankton (chloroplast-bearing cells 2-20 μm in size) in each sample were observed for ingested fluorescently labeled prey as they were encountered during the microscopical examinations. Mixotrophic nanoplankton (MNAN) were operationally defined as cells containing autofluorescent chloroplasts and ingested fluorescently labeled prey. The relative abundance of mixotrophic nanoplankton was determined as a percentage of the phototrophic nanoplankton. The percentages were then converted to absolute abundances of mixotrophic nanoplankton based on the abundances of phototrophic nanoplankton in the samples. This method for determining the abundances of mixotrophic nanoplankton is

based on two assumptions: (1) fluorescently labeled prey are ingested at the same rate as natural prey, and (2) all MNAN actively phagocytize prey during experiments. This method therefore provides a lower limit estimate of the actual abundances of mixotrophic nanoplankton present in a water sample because some mixotrophic cells may not phagocytize prey during experiments. While these latter mixotrophic individuals will be undetected by this method, at present, there is no other way to establish the presence of mixotrophs in natural samples.

Vertical distribution of mixotrophic nanoplankton during April 1992

Vertical profiles of mixotrophic nanoplankton abundance were determined at two stations south of Bermuda in the Sargasso Sea at 25°59'N, 64°30'W on April 1 and 30°26'N, 64°30'W on April 3, 1992 during R/V Endeavor cruise leg 234. Samples were collected as described above from five depths including the surface mixed layer and deep chlorophyll maximum. Duplicate 1 L subsamples from each depth were prepared and incubated as described. Fluorescently labeled bacteria (FLB) were prepared from cultures of *Pseudomonas halodurans* (~0.8-1.0 µm average cell size) according to the method of Sherr *et al.* (1987) and used as prey. FLB were added to samples at 30 to 50 percent of the natural prey abundance. The samples were incubated as described above for 6 hours starting in late afternoon. Subsamples were removed prior to incubation and at the end of the 6 hour period and preserved to establish microbial population abundances (bacteria, chroococcoid cyanobacteria, heterotrophic and phototrophic nanoplankton), the initial densities of the labeled prey and the abundance of mixotrophic nanoplankton.

Experimental examination of algal phagotrophy

An experiment was designed to determine the influence of light, nutrients and prey

density on phagotrophic behavior by algae in surface waters of the Sargasso Sea. Whole

seawater samples were subdivided and treated, and microbial populations including

mixotrophic nanoplankton were monitored for their responses to the addition of inorganic

nutrients, labile organic carbon, and changes in light intensity. Water collection was

conducted at approximately the location of the Bermuda Atlantic Time Series (BATS)

station at 31°N, 64°W. One experiment (Expt. I) was carried out between 27 and 31 March

1992 with water collected from a depth of 10 m. The experiment was repeated (Expt. II)

between 5 and 9 April 1992 using water collected from a depth of 20 m.

Water for the experiments was collected in several 0.1 N HCl-rinsed 5 L Niskin

bottles using a rosette sampler. Water was pooled in acid-rinsed carboys and subsamples

were removed and preserved with gluteraldehyde prior to filling incubation vessels in

order to establish the initial microbial population abundances. Within 1 hour of

collection duplicate clear 4 L polycarbonate vessels were filled with 3.5 L of sample and

placed in an on-deck Plexiglas incubator with running seawater to maintain water at

ambient surface water temperature. Light was adjusted with neutral density screening to

approximately 50% incident light. The experimental treatments (described below) were

incubated over a 4 day period.

C	Control, untreated seawater
G	Seawater with daily additions of glucose (10 μM C)
NP	Seawater with daily additions of NH_4^+ (5 μM) and PO_4^{-3} (0.5 μM)
GNP	Seawater with daily additions of glucose, NH_4^+ and PO_4^{-3} at above concentrations
LL	Seawater at low light intensity (<5 % incident light) without inorganic or organic additions
LLGNP	Seawater at low light intensity with daily additions of glucose, NH_4^+ and PO_4^{3-} at above concentrations

The purpose of adding glucose alone was to stimulate the bacterial assemblage and thereby cause an increase in their uptake of dissolved nutrients. This was expected to increased competition for nutrients with the phototroph assemblage. Lower concentrations of dissolved inorganic nutrients were hypothesized to favor mixotrophic algae that may be able to acquire nutrients by consumption of bacteria. Combining additions of glucose and inorganic N and P was expected to increase bacterial abundances without also causing a reduction in nutrient concentration, so that competition for nutrients would not be increased. This treatment would presumably favor mixotrophic algae that are able to take advantage of higher prey abundances. N and P were added alone to stimulate the growth of phototrophs. It was hypothesized that primarily heterotrophic species of mixotrophs and obligate photophagotrophs would continue to consume bacteria in these enclosures. Finally, the purpose of reducing light intensity was to decrease photosynthetic rates, while nutrients and glucose were added to stimulate the heterotroph assemblages in these enclosures. Mixotrophic algae that are able to supplement photosynthetic carbon by consuming bacterial C would presumably be favored in light limiting conditions, and primarily heterotrophic algae would be expected to do well in the presence of higher bacterial abundances resulting from added carbon and nutrients.

Subsamples (750 ml) were removed from each experimental treatment vessel immediately after initiation of the experiment and incubated with FLB in 1.2 L polycarbonate vessels for 6 hours. These incubations were conducted using fluorescently labeled *P. halodurans* added at a concentration equal to 50% of the natural bacterial abundance. Following these incubations, preserved samples were examined by epifluorescence microscopy, as described earlier, to establish the abundances of mixotrophic nanoplankton and other microbial assemblages. This procedure was repeated on days 2 and 4 of the 4 day experimental incubation.

RESULTS

Abundances and vertical distributions of mixotrophic nanoplankton

Labeled cyanobacteria were observed in the food vacuoles of chloroplast-containing nanoplankton in surface water samples on all seven sampling dates in August 1989 (Table 2.1). Five of these surface water samples contained detectable but low abundances of algae with ingested labeled prey (<5% of phototrophic nanoplankton). However, on 15 and 16 August, we observed abundances of 144 mixotrophic nanoplankton ml^{-1} and 70 mixotrophic nanoplankton ml^{-1} representing 53% and 50% of the total phototrophic nanoplankton in the respective surface water samples (Table 2.1). These mixotrophic nanoplankton were 2-5 μm in diameter. Considering only the phototrophic nanoplankton in this size range, 74% and 80% of the algae in the two surface water samples on 15 and 16 August contained ingested prey. These latter percentages are typical of percentages that might be observed for some species of bacterivorous heterotrophic nanoflagellate populations growing exponentially in laboratory cultures (Sieracki *et al.*, 1987; Nygaard *et al.*, 1988). This result indicates that an abundant and very active phagotrophic algal population was present in these two surface water samples. In contrast, ingested prey were not observed in any of the nanoplanktonic algae from samples collected in the deep chlorophyll maximum during August 1989.

Detailed vertical profiling conducted in early April 1992 indicated the presence of mixotrophic nanoplankton. The water column was weakly stratified during sampling in 1992 (Fig. 2.1), and there were pronounced peaks in chlorophyll concentration at 118 m on 1 April and at 132 m on 3 April. Coinciding with these deep chlorophyll maxima were peak abundances of photosynthetic nanoplankton (PNAN) of 1.2x10^3 PNAN ml^{-1} on 1 April (Fig. 2.2a) and 1.1x10^3 PNAN ml^{-1} on 3 April (Fig. 2.3a). Changes in

Table 2.1. Population abundances (±1 SD) and percentages of phototrophic nanoplankton with ingested prey determined from water samples collected from seven sampling stations in the Sargasso Sea during August 1989. "+" indicates that phototrophic nanoplankton with ingested prey were present, but at an abundance <5% of the total phototrophic nanoplankton abundance. "UN" indicates that phototrophic nanoplankton with ingested prey were undetected.

Date	Lat. Long.	Depth (m)	Bacterial Abundance ($\times 10^5$ ml^{-1})	Chroococcoid Cyanobacterial Abundance ($\times 10^3$ ml^{-1})	Phototrophic Nanoplankton Abundance ($\times 10^2$ ml^{-1})	Heterotrophic Nanoplankton Abundance ($\times 10^2$ ml^{-1})	Percent of Total Phototrophic Nanoplankton with Ingested Prey
5 Aug 89	31° 51.90' 64° 49.80'	surface	2.16±0.17	19.7±0.25	5.16±0.69	6.19±0.86	+
		100	1.55±0.21	0.34±0.17	26.1±0.76	4.60±0.64	UN
8 Aug 89	32° 07.50' 64° 19.30'	surface	2.39±0.26	10.3±0.11	3.78±1.11	5.16±1.03	+
		100	1.39±0.13	1.19±0.11	9.45±1.68	3.72±0.64	UN
10 Aug 89	31° 51.90' 64° 19.30'	surface	2.21±0.25	5.93±0.76	1.17±0.29	2.93±0.62	+
		100	1.48±0.11	0.48±0.70	1.68±0.26	3.04±0.53	UN
11 Aug 89	31° 53.58' 63° 58.80'	surface	1.71±0.24	4.42±0.54	1.11±0.28	4.96±0.92	+
13 Aug 89	32° 07.58' 64° 20.20'	surface	1.66±0.17	6.72±0.60	0.64±1.70	5.17±0.11	+
		120	1.06±0.23	0.44±1.20	1.80±0.28	2.33±0.25	UN
15 Aug 89	32° 06.80' 64° 19.91'	surface	2.05±0.29	8.94±1.47	2.72±0.56	5.35±0.60	53
		111	1.04±0.16	0.22±0.39	1.48±0.54	1.71±0.68	UN
16 Aug 89	32° 05.00' 64° 21.21'	surface	1.87±0.30	8.94±1.33	1.39±0.28	3.05±0.20	50
		120	1.09±0.18	.23±0.40	2.96±0.70	1.08±0.21	UN
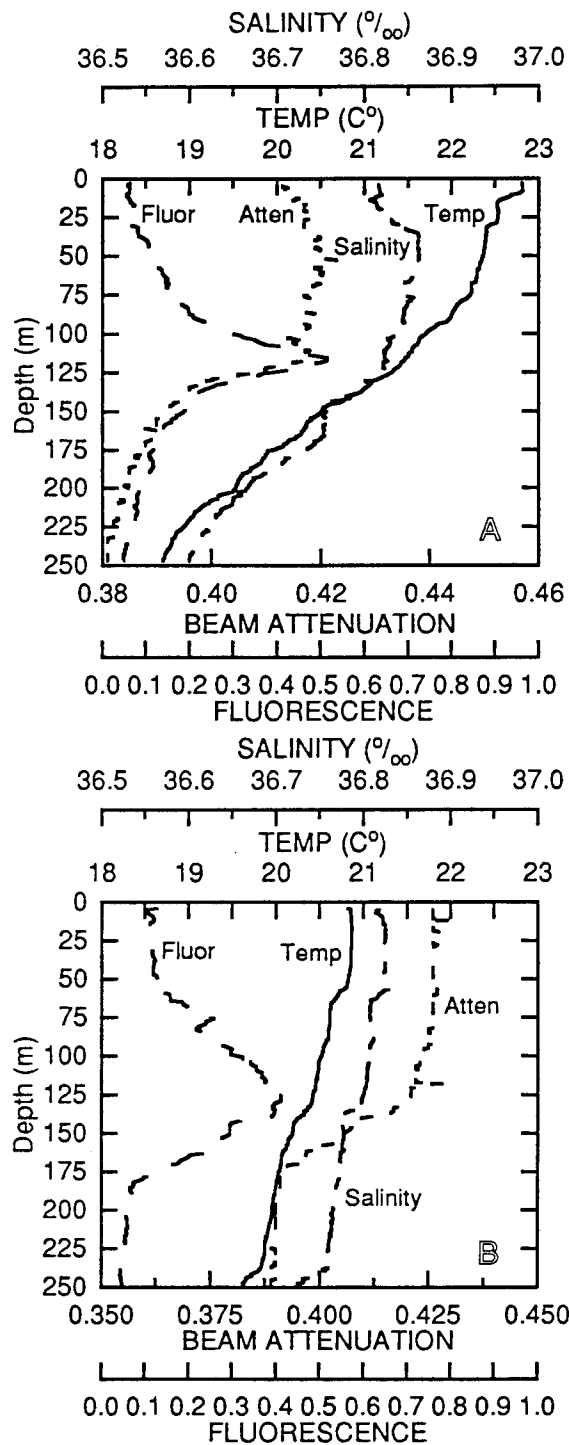
Figure 2.1. Vertical profiles of temperature, salinity, beam attenuation and fluorescence determined at 25°59'N, 64°30'W (A) sampled on 1 April 1992 and 30°26'N, 64°30'W (B) sampled on 3 April 1992.
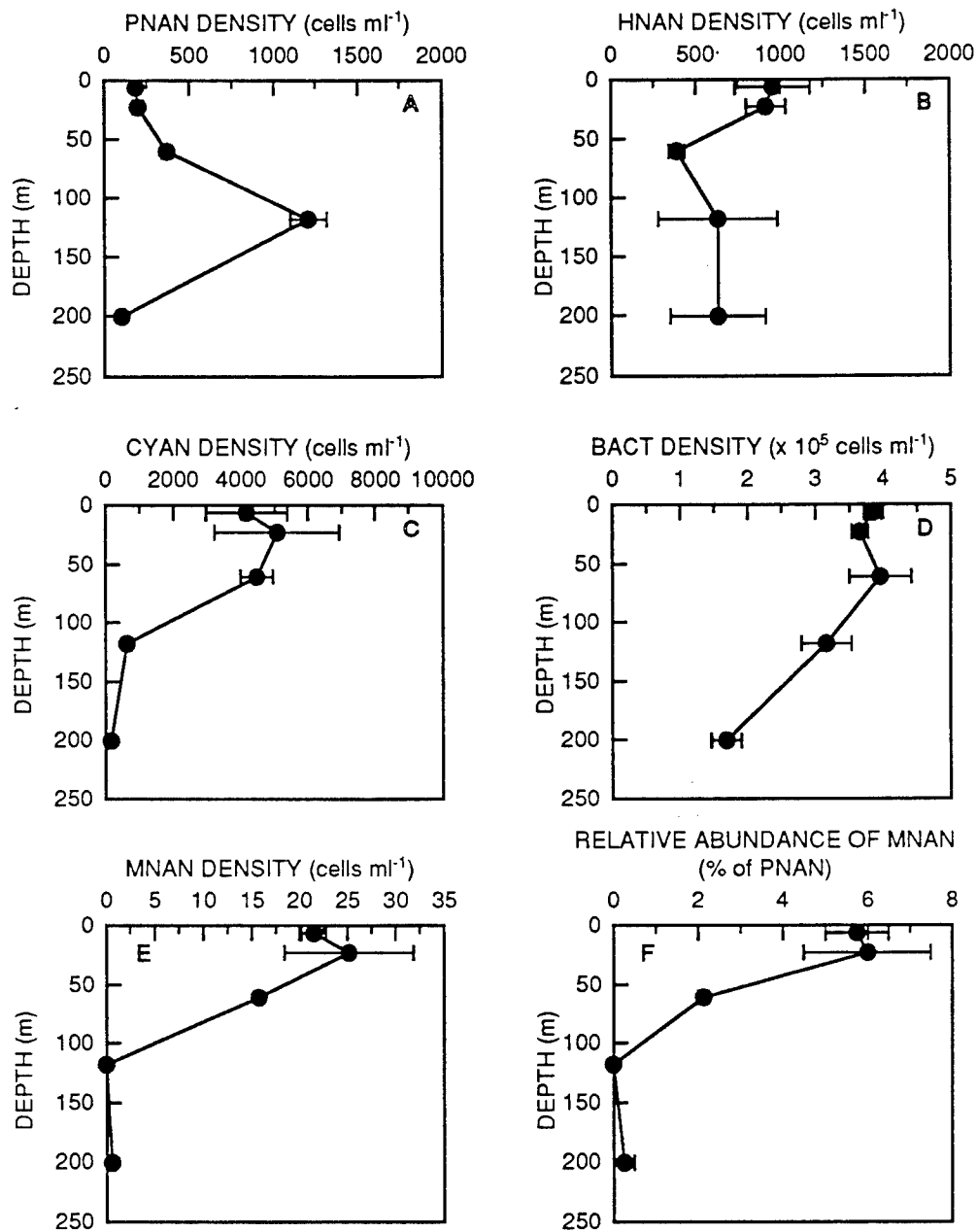
Figure 2.2. Vertical distribution of phototrophic nanoplankton (A), heterotrophic nanoplankton (B), chroococcoid cyanobacteria (C), bacteria (D) and mixotrophic nanoplankton absolute (E) and relative abundance (F) on 1 April 1992.
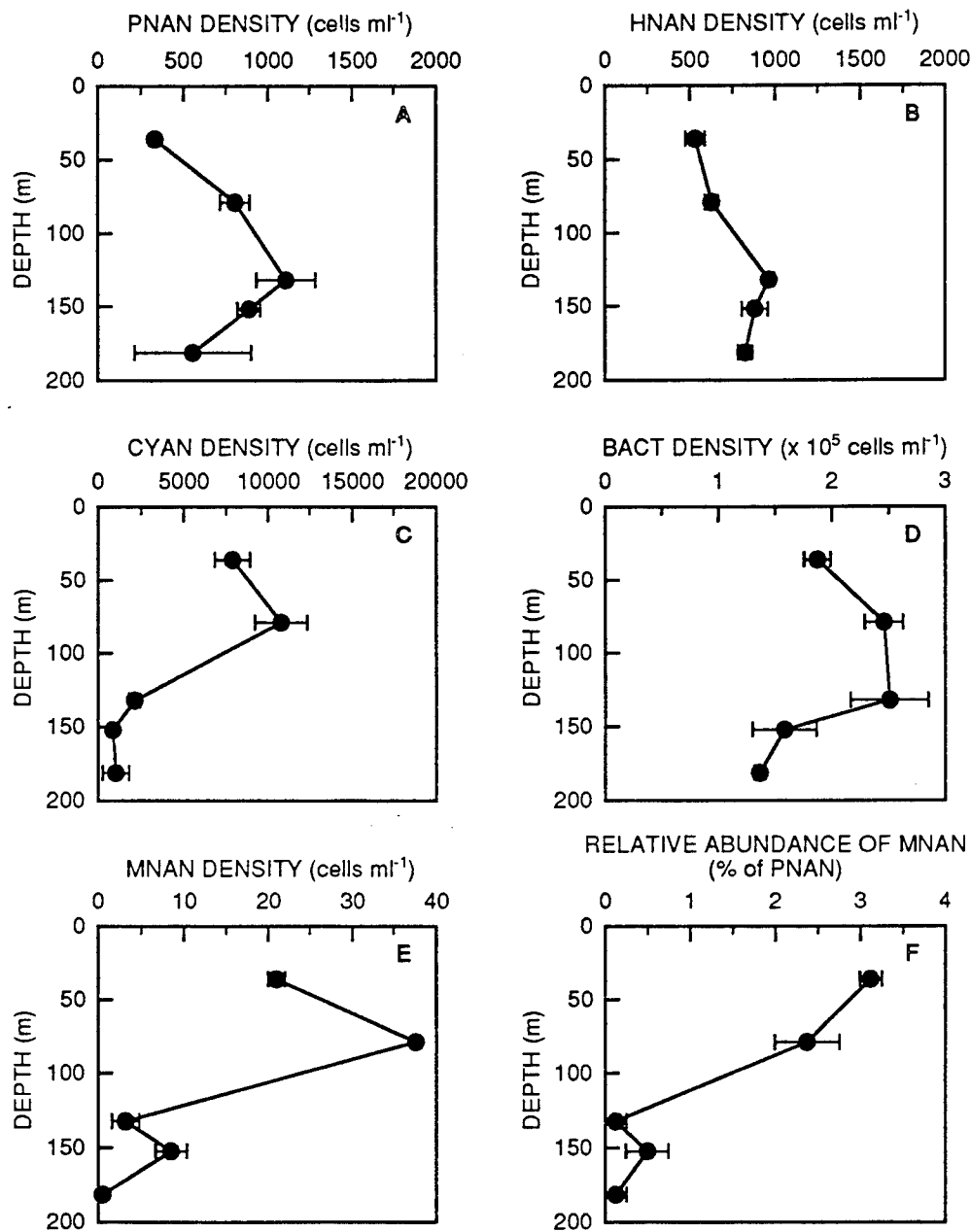
Figure 2.3. Vertical distribution of phototrophic nanoplankton (A), heterotrophic nanoplankton (B), chroococcoid cyanobacteria (C), bacteria (D) and mixotrophic nanoplankton absolute (E) and relative abundance (F) on 3 April 1992.

bacterial abundances were less dramatic than changes in PNAN abundances. Bacterial

abundances (BACT) peaked at 60 m on 1 April at 4.0×10^5 BACT ml^{-1} (Fig. 2.2d). On 3

April a maximum bacterial abundance of approximately 5×10^5 BACT ml^{-1} occurred at

132 m (Fig. 2.3d). Chroococcoid cyanobacteria (CYAN) abundances had subsurface

maxima of 5.1×10^3 CYAN ml^{-1} and 1.1×10^4 CYAN ml^{-1} at 23 m on 1 April (Fig. 2.2c)

and approximately 79 m on 3 April (Fig. 2.3c), respectively. The vertical distributions

of heterotrophic nanoplankton (HNAN) were relatively uniform during this cruise. Their

abundances ranged from 6×10^2 to 9×10^2 HNAN ml^{-1} on 1 April (Fig. 2.2b) and from

5.3×10^2 to 9.4×10^2 HNAN ml^{-1} on 3 April (Fig. 2.3b).

The relative abundance of mixotrophic nanoplankton was greatest in the

shallower water samples at both stations, but overall, the density of MNAN represented a

relatively small contribution to the total phototrophic nanoplankton assemblage at these

depths (~6%, Fig. 2.2f and <4 %, Fig. 2.3f). A maximum density of 25 MNAN ml^{-1} was

observed at 23 m on 1 April (Fig. 2.2e), and on 3 April, a maximum density of 38

MNAN ml^{-1} occurred at a depth of 79 m (Fig. 2.3e). On both sampling dates

mixotrophic nanoplankton abundances in the DCM were at or below the limit of

detection (<3 MNAN ml^{-1}).

Dissolved inorganic nutrients concentrations were low in surface waters during

the study period. According to profiles of inorganic nutrient concentrations determined

as a part of the BATS station sampling program on dates that bracketed the study period

(18 March and 12 April 1992; Fig. 2.4), nitrogen was undetectable (<0.05 μM) in surface

waters down to a depth of 40 m while phosphorus concentrations were «0.1 μM

throughout the euphotic zone. Below 40 m to approximately 200 m, dissolved inorganic

nitrogen concentrations were between 0.4 and 1.0 μM.
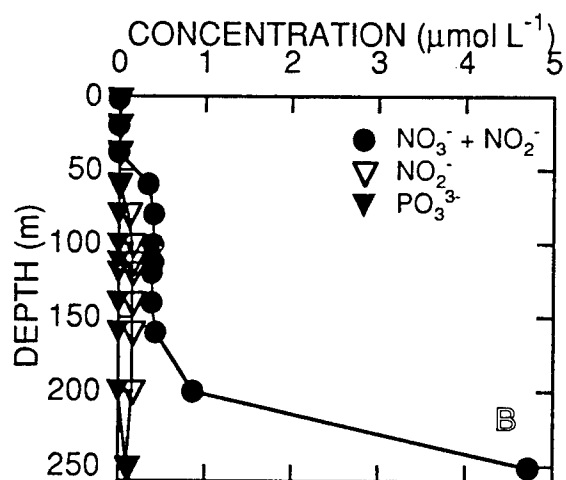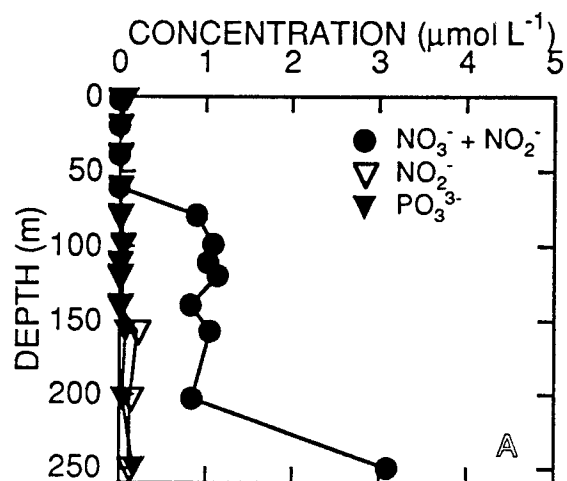
Figure 2.4. Vertical profiles of dissolved inorganic nitrogen and phosphorus concentrations at the Bermuda Atlantic Time Series station (31°N, 64°W) on 18 March 1992 (A) and 12 April 1992 (B). Data kindly provided by BATS program.

Most microbial assemblages responded to the experimental manipulations during the 4 day period. Trends generally were apparent at day 2 but were not significantly different from day 0 values for some treatments. Analyses were therefore conducted using the results from day 4, and treatments were contrasted to controls using transformed data to homogenize the variances. Significant differences were determined at the 0.05 significance level. The abundances of all microbial populations, except MNAN, did not change significantly in controls over the 4 day experimental period (Table 2.2). Absolute MNAN abundances decreased in controls during Expt. I and increased in controls during Expt. II. As a result, MNAN abundances in controls at the end of Expt. I were almost an order of magnitude lower than abundances in controls on day 4 of Expt. II even though day 0 abundances in both experiments were similar. Results from Expt. I, however, were similar to results from Expt. II with regard to the change in MNAN abundance in each treatment as compared to the control (Figs. 2.5e; 2.6e).

Bacterial abundances were significantly greater than controls on day 4 in the GNP and LLGNP treatments in both Expt. I and Expt. II (Fig. 2.5a; 2.6a). In these two treatments bacterial abundances reached values of 2×10^6 and 3×10^6 BACT ml^{-1}, respectively. Abundances in controls were $\leq 5 \times 10^5$ BACT ml^{-1}. Heterotrophic nanoplankton abundances in Expt. I did not increase significantly in the GNP or LLGNP treatments. HNAN abundances in the NP and GNP treatments, however, did appear to be slightly elevated in comparison to controls, although not significantly. These small increases may have been due to HNAN preying primarily upon members of the phototroph assemblages which increased significantly in these two treatments during Expt. I (see below). HNAN abundances in Expt. II were significantly greater than controls on day 4 in the GNP and LLGNP treatments (Fig. 2.6c). Presumably, this

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Table 2.2. Population abundances (±1 SD) in controls determined at the beginning and end of each 4 day experimental incubation. Experiment I was conducted from March 27-31 and Experiment II was conducted from April 5-9, 1992.

Experiment	Bacterial Abundance ($\times 10^5$ ml^{-1})	Chroococcoid Cyanobacterial Abundance ($\times 10^3$ ml^{-1})	Phototrophic Nanoplankton Abundance ($\times 10^2$ ml^{-1})	Heterotrophic Nanoplankton Abundance ($\times 10^3$ ml^{-1})	Mixotrophic Nanoplankton Abundance (ml^{-1})
I					
Day 0	5.02 ±0.23	7.56 ±1.28	8.16 ±2.17	1.42 ±0.34	18.84 ±5.50 *
Day 4	3.46 ±0.11	6.26 ±2.06	6.22 ±2.45	1.51 ±0.52	7.77 ±3.06 *
II					
Day 0	4.48 ±0.72	17.9 ±6.11	11.5 ±3.17	8.13 ±0.03	17.45 ±5.98 *†
Day 4	4.31 ±6.10	7.49 ±10.6	12.5 ±17.6	8.00 ±1.13	78.62 ±56.90 *

* Indicates Day 0 and Day 4 population abundances are significantly different (P-value=0.05). Significant differences were determined by contrasts performed on transformed (ln or -1/x) data.
† FLB concentration used for this initial incubation was ≈100% of natural bacterial abundance.
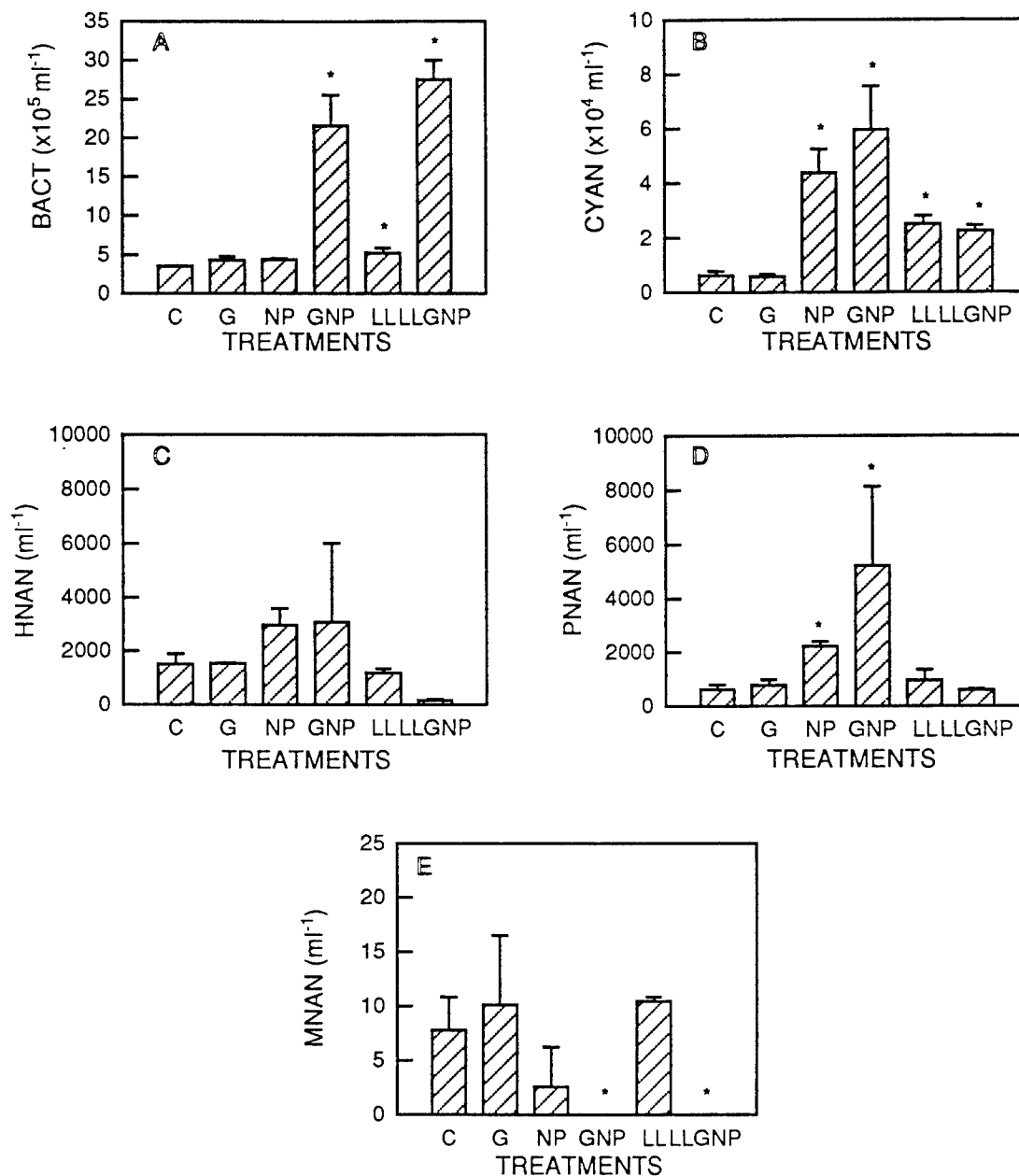
Figure 2.5. Mean population abundances (±1 SD) of bacteria (A), chroococcoid cyanobacteria (B), phototrophic nanoplankton (C), heterotrophic nanoplankton (D), and mixotrophic nanoplankton (E) in controls (C), glucose (G), nitrogen+phosphorus (NP), glucose+nitrogen+phosphorus (GNP), low light (LL) and low light with glucose+nitrogen+phosphorus (LLGNP) treatments at the end of a 4 day experimental incubation conducted from 27-31 March 1992 with water collected from a depth of 10 m at 25°59'N, 64°30'W in the Sargasso Sea. Significant differences between treatments and controls are indicated by "*" (P-value = 0.05). See text for details of treatments.
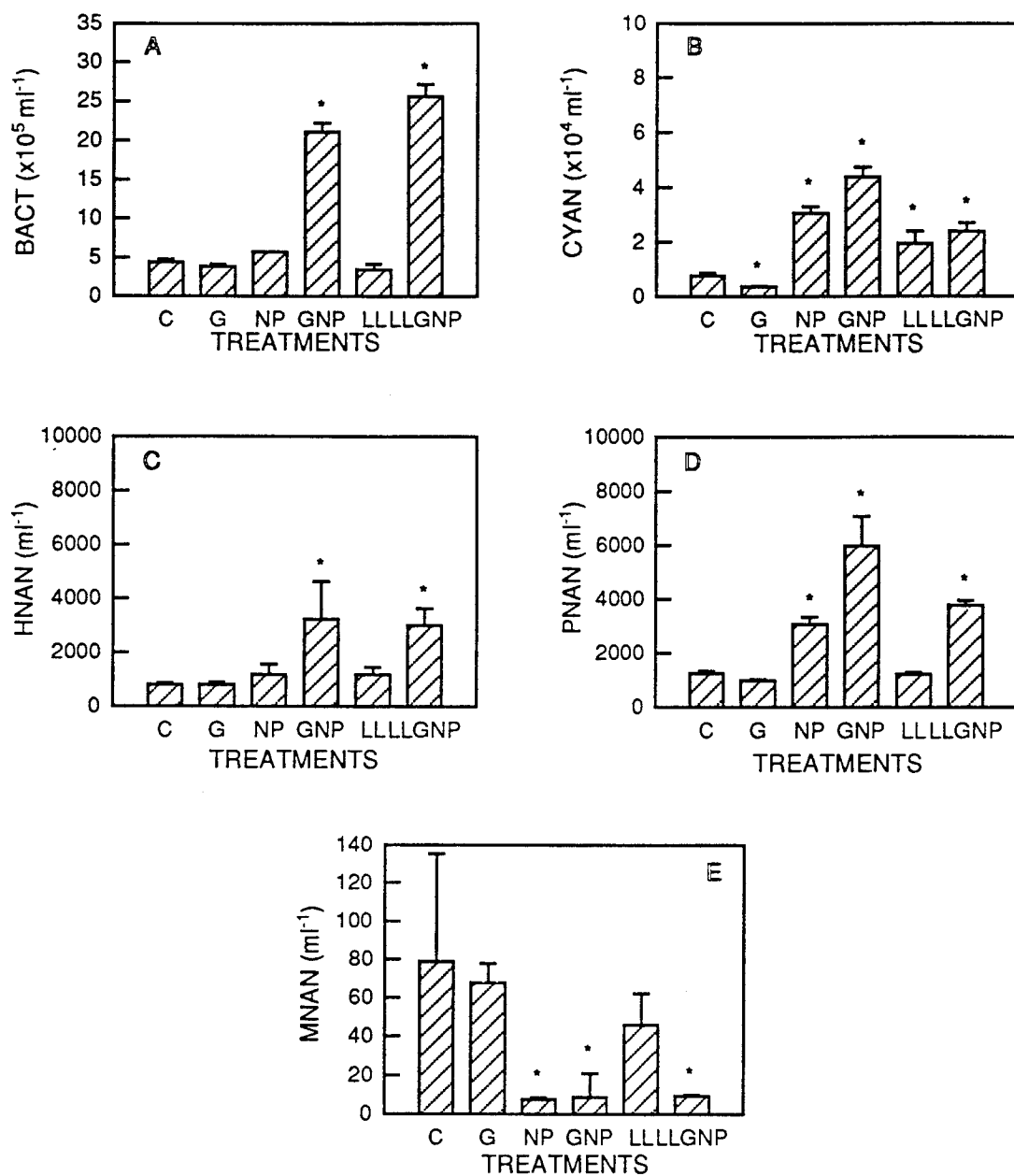
Figure 2.6. Mean population abundances (±1 SD) of bacteria (A), chroococcoid cyanobacteria (B), phototrophic nanoplankton (C), heterotrophic nanoplankton (D) and mixotrophic nanoplankton (E) in controls (C), glucose (G), nitrogen+phosphorus (NP), glucose+nitrogen+phosphorus (GNP), low light (LL) and low light with glucose+ nitrogen+phosphorus (LLGNP) treatments at the end of a 4 day experimental incubation conducted from 5-9 April 1992 with water collected from a depth of 20 m at 30°26'N, 64°30'W in the Sargasso Sea. Significant differences between treatments and controls are indicated by "*" (P-value = 0.05). See text for details of treatments.

response was due to the increase in bacterial prey abundance that also occurred in these treatments.

Abundances of cyanobacteria (CYAN) and non-phagocytizing phototrophic nanoplankton (PNAN) responded to nutrient supplementation and changes in light intensity (Fig. 2.5b, d; 2.6b, d). CYAN and PNAN abundances in treatments that received additions of inorganic nitrogen and phosphorus (NP, GNP) were significantly higher than abundances in controls. PNAN abundances were also significantly higher than abundances in controls in the LLGNP treatment on day 4 of Expt. II. Abundances of CYAN were significantly higher than abundances in controls in both low light treatments (LL, LLGNP) at the end of both experiments. CYAN abundances on day 4 ranged from $\leq 1 \times 10^4$ CYAN ml^{-1} in controls to ~5×10^4 CYAN ml^{-1} and $\geq 6 \times 10^4$ CYAN ml^{-1} in the GNP treatments in Expt. I and Expt. II, respectively. Abundances of PNAN ranged from ~1×10^3 PNAN ml^{-1} in controls to ~6×10^3 PNAN ml^{-1} in the GNP treatments at the end of Expt. I and Expt. II. These increases in the abundances of CYAN and PNAN correspond to net growth rates of approximately 0.4 d^{-1} for these assemblages. PNAN and CYAN abundances in the glucose amended treatment (G) on day 4 were not significantly increased relative controls (Expt. I ~1×10^3 PNAN ml^{-1}, Expt. II $\leq 1 \times 10^4$ CYAN ml^{-1}).

Mean MNAN abundances in controls on day 4 were ~8 ml^{-1} in Expt. I and ~80 ml^{-1} in Expt. II, and MNAN abundances in the glucose (G) and low light (LL) treatments were not significantly different from controls (Fig. 2.5e; 2.6e). Mean abundances of MNAN in the treatments that received additions of inorganic nutrients in Expt. II (NP=7.6 ml^{-1}, GNP=8.7 ml^{-1}, LLGNP=9.3 ml^{-1}), on the other hand, were significantly lower than MNAN abundances in controls at the end of Expt. II. While the same pattern was also apparent among treatments at the end of Expt. I, only the GNP and LLGNP treatments were significantly different from controls. The variances associated

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with this first experiment, however, were high due to the very low abundances of MNAN observed during this experiment.

DISCUSSION

Algal phagotrophy in surface waters of the Sargasso Sea

The results of the 4 day experimental incubations support the supposition that nutrients limited algal growth in surface waters at the time of the study. Cyanobacteria and phototrophic nanoplankton abundances increased substantially (by a factor of 5-6) in treatments that were enriched with inorganic nutrients (NP, GNP, LLGNP). Their abundances did not change in the controls (C) or the glucose amended treatment (G). PNAN also did not increase in the low light treatment (LL). The heterotrophic bacterial population experienced no significant increases in abundance with the addition of inorganic nutrients (NP) or glucose (G) added separately, but they experienced a large increase when inorganic nutrient enrichment was accompanied by additions of labile organic carbon (GNP, LLGNP). These results indicate that bacterial growth was co-limited (or close to co-limitation) by inorganic nutrients and organic carbon supply.

In contrast to the phototrophs, mixotrophic nanoplankton were less abundant in nutrient enriched treatments (NP, GNP, LLGNP) at the end of the 4 day experimental incubations and more abundant in treatments that were nutrient limited (C, G, LL). Based on these results, we suggest that phagotrophy may provide mixotrophic algae in surface waters of the Sargasso Sea with major nutrients. During periods of low nutrient concentrations phagotrophic behavior may enable mixotrophic algae to survive in competition with purely phototrophic algae. When dissolved nutrient concentrations are increased, though, mixotrophic algae may be out-competed by other phototrophs. This scenario could account for the low abundances of mixotrophs in nutrient enriched treatments.

On the other hand, while mixotrophic algae present in surface waters of the Sargasso Sea may satisfy nutrient requirements via phagotrophy during periods of

nutrient limitation, they may be capable of abandoning this behavior in favor of phototrophic acquisition of dissolved nutrients when dissolved nutrient concentrations are increased. Mixotrophs not actively engaged in phagotrophy would not be detectable by the FLB uptake method that we employed. Decreases in mixotroph abundances with nutrient enrichment could therefore have been due to a cessation in phagotrophic activity by mixotrophic algae rather than actual decreases in their abundances. Support for this alternative explanation is found in a study by Nygaard and Tobiesen (1993) who examined bacterivory by several species of marine mixotrophic flagellates in P-limited and non-limited conditions. They observed that bacterial ingestion rates by some species were higher under conditions of P-limited phototrophic growth. Similarly, a field study conducted in a chrysophyte-dominated freshwater lake containing large numbers of phagotrophically active *Dinobryon* spp., indicated that the percent of *Dinobryon* cells with ingested prey decreased when dissolved nutrients were added, but the actual number of *Dinobryon* cells increased provided there was adequate light for photosynthesis (Chapter 4). Nutrient limitation, whether it influences cell numbers or phagotrophic activity, may explain the observations of higher mixotroph abundances in surface waters during this study.

Mixotrophic species in which phagotrophy serves different purposes may have been present in water samples at the time of this study, but our data do not support this contention. For example, it is possible that mixotrophic algae deficient in their ability to synthesize a required growth compound, similar to *Uroglena americana* (Kimura & Ishida, 1989), were present in surface waters. Rather than acquiring major nutrients, phagotrophic behavior may be necessary for these algae to obtain an essential growth factor. Mixotrophs with this growth strategy, however, would be expected to respond favorably to increased prey abundance. Yet, increases in mixotroph abundances were not observed in treatments that had increased bacterial abundances (GNP, LLGNP).

Based on this information we conclude that mixotrophic species displaying this trophic behavior were probably not important in surface waters at the time of this study.

Mixotrophic species that are primarily heterotrophic and satisfy most of their carbon and nutrient requirements by phagotrophy were also probably not important contributors to the mixotroph assemblage in surface waters at the time of this study. Mixotrophs with this growth strategy would have been expected to increase in abundance in both low light treatments (due to a competitive advantage under poor phototrophic conditions, and/or the treatment with added carbon and nutrients (due to increased prey abundance). Perhaps low bacterial abundances in the low light treatment without any additions prevented an increase in their abundance in this treatment, but this should not have been the case in the low light treatment with added glucose, N and P, or in the ambient light treatment with added glucose, N and P. Abundances of mixotrophic nanoplankton in these treatments were not significantly different from abundances in controls even though bacterial abundances in these treatments were above $1 \times 10^6 \text{ ml}^{-1}$, the reported grazing threshold for species with similar growth strategies (Sanders *et al.*, 1990).

A potential source of bias that may possibly account for the decrease in MNAN abundances in the two treatments enriched with C, N and P (GNP & LLGNP), though, is the ratio of fluorescently labeled prey to natural bacteria used in the FLB uptake experiments to examine ingestion by phototrophic nanoplankton. Increased numbers of bacterial prey in the GNP and LLGNP treatments would have been expected to increase the competitive capabilities of mixotrophic species that are primarily heterotrophic. However, because all of the 6 hour FLB incubations were conducted with the same absolute abundance of FLB added, the FLB:bacteria ratio on day 4 was 5 times lower in the GNP and LLGNP treatments than in the other treatments. Some actively feeding mixotrophs may not have contained any ingested FLB as a result, and the actual number

of MNAN may have been underestimated in these two treatments. We investigated this potential bias using ingestion and digestion rates taken from the literature, as follows.

The ratio of FLB to natural bacteria in the treatments receiving additions of glucose, nitrogen and phosphorus (GNP and LLGNP) was approximately 1:10. In the other treatments (C, G, NP, LL), it was approximately 1:2. Average ingestion rates for mixotrophic nanoplankton range from 5-103 bacteria cell^{-1} h^{-1} (Bird & Kalff, 1987; Caron et al., 1990; Caron et al., 1992). Digestion times for phagotrophic protozoa are reported to be between 30-60 minutes (Sherr et al., 1988; Jones et al., 1993). Assuming a conservative ingestion rate of 20 bacteria cell^{-1} h^{-1} and a digestion time of 30 minutes, we calculated that there should have been at least 1 ingested FLB per actively feeding mixotroph in the GNP and LLGNP treatments at any given time and at least 5 FLB per actively feeding mixotroph in the other treatments (C, G, NP, LL). It is therefore unlikely that the ratio of FLB to natural bacteria lead to an underestimation of mixotroph abundance in the GNP and LLGNP treatments in either experiment.

MNAN abundances may, however, have been underestimated to some degree in nutrient enriched treatments due to a reduction in the ability to detect MNAN as a result of increased PNAN abundances in these treatments. The limit of detection for MNAN can be calculated from the number of PNAN examined for ingested FLB (400) and total PNAN abundance. The limit of detection for the treatments in which abundances of PNAN were not significantly different from controls was ≤3 MNAN ml^{-1}. On the other hand, the limits of detection for treatments with significantly increased abundances of PNAN ranged from approximately 5 MNAN ml^{-1} in the NP treatment in Expt. I to 15 MNAN ml^{-1} in the GNP treatment in Expt. II. Thus, actual abundances of MNAN may have been as high as 5 ml^{-1} in the NP treatment and 12 ml^{-1} in the GNP treatment in Expt. I, and as high as 7 ml^{-1} in the NP treatment, 15 ml^{-1} in the GNP treatment and 10 ml^{-1} in the LLGNP treatment in Expt. II and still have gone undetected. Because abundances of MNAN in controls at the end of Expt. I were <10 ml^{-1}, MNAN

abundances in the NP and GNP treatments in this experiment may not have been significantly different from MNAN abundances in controls. On the other hand, overall abundances of MNAN at the end of Expt. II were almost an order of magnitude greater than MNAN abundances at the end of Expt. I (nearly 80 ml^{-1} in controls). Therefore, even if MNAN abundances in the NP, GNP and LLGNP treatments in Expt. II were as high as the calculated limits of detection, they would still have been significantly lower than MNAN abundances in controls.

Vertical distribution of mixotrophic nanoplankton in euphotic zone waters of the Sargasso Sea

Based on the experimental results, it can be hypothesized that the mixotroph assemblage in surface waters of the Sargasso Sea during this study was dominated by mixotrophic nanoplankton that satisfy some or all of their nutrient requirements via phagotrophy, and nutrient availability may therefore account for the vertical distribution of mixotrophic nanoplankton. High abundances of mixotrophic nanoplankton were observed in surface waters, but near the base of the euphotic zone, mixotrophic nanoplankton were nearly undetectable. Phagotrophic behavior by algae in oligotrophic surface waters may be competitively advantageous, even if phagotrophic acquisition of nutrients is energetically expensive relative to the uptake of dissolved nutrients. This strategy for nutrient acquisition may not be competitive in high nutrient environments, though, and may account for low abundances of mixotrophs near the base of the euphotic zone where nutrient concentrations were high. Alternatively, mixotrophs may have been just as abundant near the base of the euphotic zone as in surface waters, but they may have reduced or ceased phagotrophic activity in response to high concentrations of dissolved nutrients.

Another possible explanation that may also account for lower abundances of mixotrophs near the base of the euphotic zone is that mixotrophic algae may be light limited at these deeper depths. Some species of mixotrophic algae are incapable of continued growth at low light intensity (Caron *et al.*, 1992). Perhaps mixotrophic algae present in euphotic zone waters of the Sargasso Sea require high light for growth. If so, mixotrophic algae may have been simply out-competed near the base of the euphotic zone by other phototrophs better adapted for growth at low light intensity.

While reasons for the observations of low abundances of MNAN near the base of the euphotic zone remain unclear, evidence has been provided in this paper to suggest that phagotrophic behavior by mixotrophic algae in surface waters of the Sargasso Sea was a consequence of the generally low nutrient concentrations at these depths. On two occasions, high abundances of mixotrophs were observed in surfaces waters. It is hypothesized that phagotrophy may permit mixotrophic algae to survive in competition with other phototrophs by providing them with a supplemental source of nutrients. This behavior may, on occasion, allow mixotrophic algae to become important contributors to the nanoplankton assemblage in oligotrophic surface waters of the Sargasso Sea.

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CHAPTER 3

Distribution and Abundance of Mixotrophic Nanoplankton in Nine
Adirondack Seepage Ponds and Three Coastal Salt Ponds

ABSTRACT

The vertical distributions and abundances of mixotrophic nanoplankton were documented along with other physical, chemical and biological parameters in nine freshwater seepage ponds in the Adirondack Mountains of New York during summer, 1992 and in three coastal salt ponds in Falmouth, Massachusetts, during summer, 1993. The nine freshwater ponds represented a gradient in dissolved organic carbon (DOC) concentration that was hypothesized to influence the distribution and abundance of mixotrophic nanoplankton in these ponds. No clear relationships existed between mixotrophic algal abundance in the Adirondack seepage ponds and the physical, chemical or biological parameters measured. Mixotrophic algae were numerically unimportant in most seepage ponds (<5% of algal nanoplankton). However, 25% of the total nanoplanktonic algal assemblage was phagotrophic in the upper water column of one pond with moderate concentrations of DOC, and absolute abundances of mixotrophic algae in the metalimnion of this pond were high ($\sim 2 \times 10^4$ ml^{-1}). *Dinobryon* was the most important contributor (~85%) to the mixotroph assemblage in the metalimnion of this mid DOC pond with peak abundances of 1.7×10^4 ml^{-1}, although only ~50% of this population ($\sim 9 \times 10^3$ ml^{-1}) and the *Dinobryon* population in the epilimnion ($\sim 1 \times 10^3$ ml^{-1}) were phagotrophically active. Mixotrophic algae were moderately important (~15%) in the upper water column of two other ponds with high concentrations of DOC, but mixotroph abundances did not exceed 1×10^3 ml^{-1}.

Documentation of the abundance of mixotrophic nanoplankton in the three coastal salt ponds provide the first evidence for their existence in small semi-enclosed coastal lagoons. Mixotrophic nanoplankton abundances were <150 ml^{-1} in all three coastal salt ponds examined, and they represented <2% of the total nanoplanktonic algal assemblage. While mixotrophy was not common among the nanoplankton assemblage in these small coastal lagoons at the time of study, mixotrophy was prevalent in the microplankton (20-

200 μm) assemblage in at least one pond. Mixotrophic (chloroplast-bearing) ciliates were present in high abundances (4×10^4 to $>1 \times 10^5$ L^{-1}) throughout the upper water column in one coastal salt pond. The abundance of these "green" ciliates relative to heterotrophic ciliates was highest in surface waters, but their absolute abundances were highest in the metalimnion.

It is clear from this study and others that mixotrophic nanoplankton can be numerically important in some freshwater environments. Interestingly though, mixotrophic nanoplankton abundances in the small coastal lagoons were consistently low during this study. High abundances of microplanktonic mixotrophs in at least one of the coastal ponds, however, suggests that mixotrophy may, on occasion, be more prevalent among the microplankton assemblages in estuarine environments. Energy and nutrient cycling may therefore differ in these environments relative to other marine and some freshwater environments where mixotrophy in the nanoplankton assemblage can be more common.

INTRODUCTION

Nanoplanktonic algae (unicellular algae 2-20 μm in size) are important
contributors to the phytoplankton assemblage in aquatic environments and are
responsible for a significant fraction of primary production (Durbin *et al.*, 1975;
Throndsen, 1976; Hallegraeff, 1981; Beers *et al.*, 1982; Ilmavirta, 1983). The algal
nanoplankton are typically dominated by members of the Chrysophyceae,
Prymnesiophyceae, Cryptophyceae, and Dinophyceae (Ilmavirta, 1983; Estep *et al.*,
1984). Interestingly, each of these flagellate taxa contains several pigmented forms that
are capable of phagotrophy in addition to photosynthesis (Sanders & Porter, 1988). This
ability to combine autotrophic and heterotrophic modes of nutrition is known as
mixotrophy.

Numerous anecdotal accounts of mixotrophy, from as early as 1911, exist in the
literature (Sanders & Porter, 1988 for a summary of references). Until recently though,
the ecological implications of algal phagotrophy in aquatic systems have been largely
ignored. However, with recognition of the importance of microheterotrophic processes
in energy and nutrient flow through planktonic food webs (Pomeroy, 1974; Azam *et al.*,
1983) and methodological developments in the area of microbial ecology (Porter & Feig,
1980; Caron, 1983; Sherr & Sherr, 1983; Sherr *et al.*, 1987), ecologists are now
beginning to evaluate the role of mixotrophs in aquatic systems.

The nutritional benefits gained from phagotrophy and the conditions under which
phagotrophy is employed by algal nanoplankton varies among mixotrophic species.
Some mixotrophic species are primarily heterotrophic and rely on phagotrophy to obtain
most or all of the organic carbon used for their metabolism and growth (Fenchel, 1982a;
Andersson *et al.*, 1989; Sanders *et al.*, 1990). Photosynthesis in these species appears to
be used as a mechanism for survival only under poor heterotrophic conditions (*i.e.*, low
prey abundance). Other species appear to be obligate mixotrophs (Kimura & Ishida,

1986; Caron *et al.*, 1992). In some of these mixotrophic species phagotrophy may serve as a source of specific growth factors required for phototrophic growth (Kimura & Ishida, 1989). It has also been suggested that phagotrophic behavior may provide some mixotrophic algae with major nutrients (nitrogen or phosphorus) which may be an especially useful strategy during periods of nutrient limitation (Salonen & Jokinen, 1988). Evidence from several recent studies have provided support for this hypothesis (Nygaard & Tobiesen, 1993, see also Chapters 2 & 4).

The potential impact of algal phagotrophy in some systems has been evaluated based on field and laboratory studies. Ingestion rates for some mixotrophic nanoplankton measured in the laboratory have been found to be comparable to ingestion rates of heterotrophs (Fenchel, 1982b; Sherr *et al.*, 1983), and mixotrophic algae have been found to dominate the nanoplankton assemblage on occasion in some aquatic environments (Bird & Kalff, 1986; Bird & Kalff, 1987; Porter, 1988; Andersson *et al.*, 1989; Bennett *et al.*, 1990; Sanders *et al.*, 1990). On occasion then, the overall community-level importance of algal phagotrophy has been great, and algal bacterial consumption has been found to exceed consumption by the heterotrophs in some of these plankton communities. For example, in Lake Memphremagog, Vermont-Quebec, ecologists found that the algal phagotroph *Dinobryon* removed more bacteria from the water column than the crustaceans, rotifers and ciliates combined (Bird & Kalff, 1986). In another study conducted in surface waters of Lake Oglethorpe, Georgia, grazing by the autotrophic community was found to constitute more than 50% of total bacterial grazing (Porter, 1988; Sanders *et al.*, 1989).

While these studies have begun to characterize the role of mixotrophic nanoplankton in several aquatic environments, the extent of their distribution and abundance in nature is unclear (Berninger *et al.*, 1992, Chapter 2). This study provides documentation of the distribution and abundance of mixotrophic nanoplankton in a series of nine freshwater seepage ponds in the Adirondack Mountains, NY, and three coastal

salt ponds in Falmouth, MA, and examines potential relationships between mixotroph abundance and other physical, chemical and biological characteristics of these aquatic systems. The occurrence of mixotrophic nanoplankton in the freshwater seepage ponds was variable and did not appear to be directly related to specific physical, chemical or biological parameters. Mixotrophic nanoplankton abundances were present at high abundances in one freshwater seepage pond, and they accounted for a significant fraction of the algal nanoplankton assemblage. Abundances of mixotrophic nanoplankton were uniformly low in all three coastal salt ponds at the time of study and their contribution in each pond to total abundances of algal nanoplankton was very low.

METHODS

Study sites

This work was carried out in nine freshwater seepage ponds in the northwest and west central regions of the Adirondack Mountains of New York during July 1992 and in three coastal salt ponds located adjacent to Vineyard Sound in Falmouth, Massachusetts, during early summer (mid June through late July) 1993. Each freshwater and coastal pond was surveyed once during the study period and the vertical distribution and abundance of phagotrophic algae 2-20 µm in size was determined in water samples collected from the surface mixed layer, thermocline and bottom layer at a single station located over the deep basin in each pond. Sampling at each site also included physical profiling, measurement of nutrient concentrations and collection of samples for the determination of microbial population abundances. Water column samples were collected using a 4 L Van Dorn bottle in the Adirondack seepage ponds and a hand vacuum pump in the Falmouth coastal ponds. Detailed descriptions of the sampling protocols are described below.

Physical characterization of ponds

Irradiance and temperature profiles for the Adirondack seepage ponds were determined on the day of sampling prior to collection of water samples. Measurements were made at 0.5 or 1 meter intervals from surface to bottom. Irradiance was measured with a Protomatic photometer equipped with upward and downward spherical sensors exposed as hemispheres, and was expressed as a percentage of light energy just below the surface. Temperature profiling was conducted using a YSI model 35 S-C-T meter. The epilimnion, metalimnion and hypolimnion were defined according to the temperature

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structure of the water column (Wetzel, 1975), and served as the basis for determining water column depths for microbial population sampling. Irradiance, temperature and salinity profiles in the Falmouth coastal ponds were determined at 0.5 m intervals prior to water column sampling. Irradiance was measured with a 4π sensor and expressed as a percentage of subsurface light. Temperature and salinity were measured using a YSI model 33 S-C-T meter. Five depths for water column sampling (chlorophyll, nutrients and microbial populations) in each coastal pond were then determined based on their temperature and salinity profiles.

Determination of dissolved oxygen

Five to six depths were sampled for dissolved oxygen in the Adirondack seepage ponds. Water column samples were fixed in 300 ml BOD bottles at each site, returned to the lab and analyzed using the azide modification of the Winkler titration method (Wetzel, 1991). Concentrations were then converted to % O_2 saturation. A temperature adjustable YSI O_2 meter, model 51b was used in the Falmouth coastal ponds to obtain profiles of dissolved oxygen concentration with measurements at 0.5 m intervals. These dissolved oxygen values were converted to % O_2 saturation based on the theoretical concentration for 100% saturation at the sampling temperature and salinity (Endeco Vapor Program).

Pigment analyses

Water samples for pigment concentrations were collected from five to eight depths in the Adirondack seepage ponds and placed in the dark prior to their return to the laboratory (<2 h). Samples were immediately processed back in the lab according to methods outlined in Bukaveckas (1993). Briefly, samples were filtered through Gelman

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A/E glass fiber filters (2-3 L sample^{-1}). Filters were macerated and allowed to extract in 10 ml of aqueous acetone (90%) for 12 h in the dark at 4°C and then analyzed using a Varion DMS 70 spectrophotometer equipped with 4 cm cells and a narrow bandwidth (1 nm). Chlorophyll *a*, and phaeophytin concentrations were determined according to the equations of Jeffrey and Humphrey (1975) for mixed phytoplankton populations and the Lorenzen equations as revised by Speziale, *et al.* (1984).

Water column samples collected from the Falmouth coastal ponds for analysis of chlorophyll, carotenoid and phaeopigment concentrations were immediately placed in cold, dark storage and then returned to the laboratory within two hours. After return to the laboratory, samples were immediately filtered and extracted according to the methods of Parsons *et al.* (1984). Extractions were analyzed with a Bausch & Lomb Spectronic 2000 Spectrophotometer and pigment concentrations were determined using spectrophotometric equations (Parsons *et al.*, 1984).

Nutrient analyses

Concentrations of dissolved inorganic nitrogen (NO_3^-, NH_4^+) and phosphorus (PO_4^{-3}) in the Adirondack seepage ponds were determined from filtered (0.45 μm glass fiber filters), acidified (pH 2.0) water samples collected from a single depth in the epilimnion and a single depth in the hypolimnion. Unfiltered samples from these depths were also collected for analysis of total phosphorus (TP). Samples were stored in the dark at 4°C and transported to the Institute of Ecosystems Study Inorganic Laboratory in Millbrook, NY, where they were analyzed. TP analysis was performed according to the molybdenum blue method of Murphy and Riley (1962) after persulfate oxidation. Phenate colorimetric analysis of NH_4^+ was performed by autoanalysis and PO_4^{-3} was analyzed by the molybdenum blue method. NO_3^- was analyzed by ion exchange chromatography (Small *et al.*, 1975).

Dissolved inorganic nitrogen (NO_3^-, NH_4^+) and phosphorus (PO_4^{-3}) concentrations in each Falmouth coastal pond were determined from water samples that were filtered through 0.22 μm Millipore filters immediately upon collection, transported (cold, dark) to the lab and frozen. Prior to analyses samples were thawed to room temperature. Dissolved inorganic phosphate was determined according to the molybdenum blue method of Murphy and Riley (1962). Determination of ammonium was by the indophenol method described by Schiener (1976), and nitrate analyses were done according to Lachat Autoanalysis procedures adapted from techniques in Wood *et al.* (1967) and Bendschneider (1952).

Determination of microbial population abundances

Microbial population abundances were determined from duplicate samples collected from one or two depths in the epilimnion, one or two depths in the metalimnion and a single depth in the hypolimnion of each Adirondack seepage pond and from the five sampling depths in each coastal pond. Subsamples from each depth were preserved immediately with gluteraldehyde at a final concentration of 1% for microscopical determination of bacterial abundances (<2 μm), and phototrophic and heterotrophic nanoplankton abundances (algae and protozoa 2-20 μm in size, respectively). Microplankton (algae and protozoa 20-200 μm in size, respectively) abundances were also determined in samples from one coastal pond after observations revealed the presence of chloroplast-retaining ciliates in that pond. Population counts were performed by epifluorescence microscopy at 1000x magnification for bacteria and nanoplankton and 400x magnification for microplankton (Porter & Feig, 1980; Sherr *et al.*, 1993).

Phagotrophic algae were identified in water samples using fluorescently labeled prey as tracers for ingestion. Duplicate water samples from each depth were incubated in clear polycarbonate vessels (250 ml, seepage ponds; 1 L coastal ponds) with fluorescently

labeled bacteria (FLB) prepared from cultures of *Pseudomonas halodurans* (~0.8-1.0 μm average cell size) according to the method of Sherr *et al.* (1987). Each vessel was inoculated with FLB within 30 minutes of collection, gently swirled to distribute the tracer and incubated *in situ* for five hours (between 9am and 4pm) at the depth from which they were collected. The density of FLB used in the experiments was chosen such that the tracers could be readily counted, but did not dramatically increase the abundance of natural prey (generally 30-50% of the natural prey abundance). At the end of the five hour incubation period a final subsample was removed and preserved with 0.05% $NiCl_2$ followed by 1% gluteraldehyde (final concentrations) for determination of mixotroph abundance. $NiCl_2$ was added to freshwater samples to prevent possible egestion of the tracers, but not to coastal pond samples because $NiCl_2$ causes cells in seawater to clump (J.B. Waterbury, WHOI, pers. comm.).

Two hundred to four hundred individual phototrophic nanoplankton (chloroplast-bearing cells 2-20 μm in size) in each sample were examined by epifluorescence microscopy for ingested fluorescently labeled prey. Mixotrophic nanoplankton (MNAN) were operationally defined as cells containing autofluorescent chloroplasts and ingested fluorescently labeled prey. The relative abundance of mixotrophic nanoplankton was determined as a percentage of the phototrophic nanoplankton. These percentages were then converted to absolute abundances of mixotrophic nanoplankton based on the abundances of phototrophic nanoplankton in the samples. This method for determining the abundance of mixotrophic nanoplankton was based on two assumptions: (1) fluorescently labeled prey were ingested at the same rate as natural prey, and (2) all MNAN actively phagocytized prey during experiments. This method therefore provided a lower limit estimate of the actual abundance of mixotrophic nanoplankton present in water samples since some mixotrophic cells may not have phagocytized prey during experiments. At present there is no other way to establish the presence of mixotrophs in natural samples.

Correlation coefficients and their *t* statistics for the freshwater seepage ponds were determined for all pairwise variables from each pond. Significant relationships between pairs of variables reported in the text are based on a test of H_0: $r = 0$ using the two-sided *t* test with *n*-2 degrees of freedom at *a* level = 0.05.

RESULTS

Freshwater Seepage Ponds

The nine freshwater seepage ponds chosen for this study were previously found to represent a gradient in dissolved organic carbon (DOC) concentration (Saunders, 1992, See Table 3.1 for site descriptions). The low DOC (<3 mg L^{-1}) and mid DOC (5-7 mg L^{-1}) ponds in this study were typified by circum-neutral pH values between 5.5 and 6.25, while high DOC ponds (15-24 mg L^{-1}) were generally more acidic with pH values slightly greater than 4 (Saunders, 1992).

Physical characteristics

Saunders (1992) found that epilimnetic mixing depths were more shallow in the ponds with high DOC concentrations. Thermal stratification was evident in eight of the nine seepage ponds at the time of this study and the shallowest epilimnetic mixing depths were also associated with high DOC ponds (Fig 3.1). Round Pond (low DOC) was well mixed to the bottom (Fig. 3.1a). Epilimnetic mixing extended down to approximately 4 m in the two weakly stratified low DOC ponds (Fig. 3.1b, c), to 2 m in the mid DOC ponds (Fig. 3.1d-f) and to 0.5-1 m in the high DOC ponds (Fig. 3.1g-i).

Light attenuation rates in these ponds were previously found to be related to epilimnetic DOC concentration (Saunders, 1992). This relationship was apparent during this study. Light intensity at the bottom of Round Pond was >5% of subsurface irradiance (Fig. 3.2a). The depth of 1% subsurface light in the other two low DOC ponds occurred below the base of the thermocline (Deer Pond, 8m, Fig 3.2b; Pitchfork Pond, 6m, Fig. 3.2c). The depth of 1% subsurface light also corresponded with the base of the

Table 3.1. Freshwater seepage pond and coastal salt pond surface areas, basin depths and dissolved organic carbon concentrations (freshwater ponds only). Information for freshwater seepage ponds taken from Saunders (1992). Data for coastal salt ponds taken from Giblin (1990).

Site Name	Surface Area (ha)	Basin Depth (m)	DOC (mg L^{-1})
Freshwater Seepage Ponds			
Low DOC			
Round	4	7	0.1
Deer	10	14	1.4
Pitchfork	16	8	2.8
Mid DOC			
Wheeler	6	18	5.7
Kanacto	4	15	6.9
Rat	12	9	6.9
High DOC			
Amphitheater	1	6	24.5
Fox	1	10	15.1
Little Echo	1	4	18.8
Coastal Salt Ponds			
Perch	7	3	--
Oyster	25	4	--
Salt	29	6	--

Figure 3.1. Vertical profiles of temperature in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.
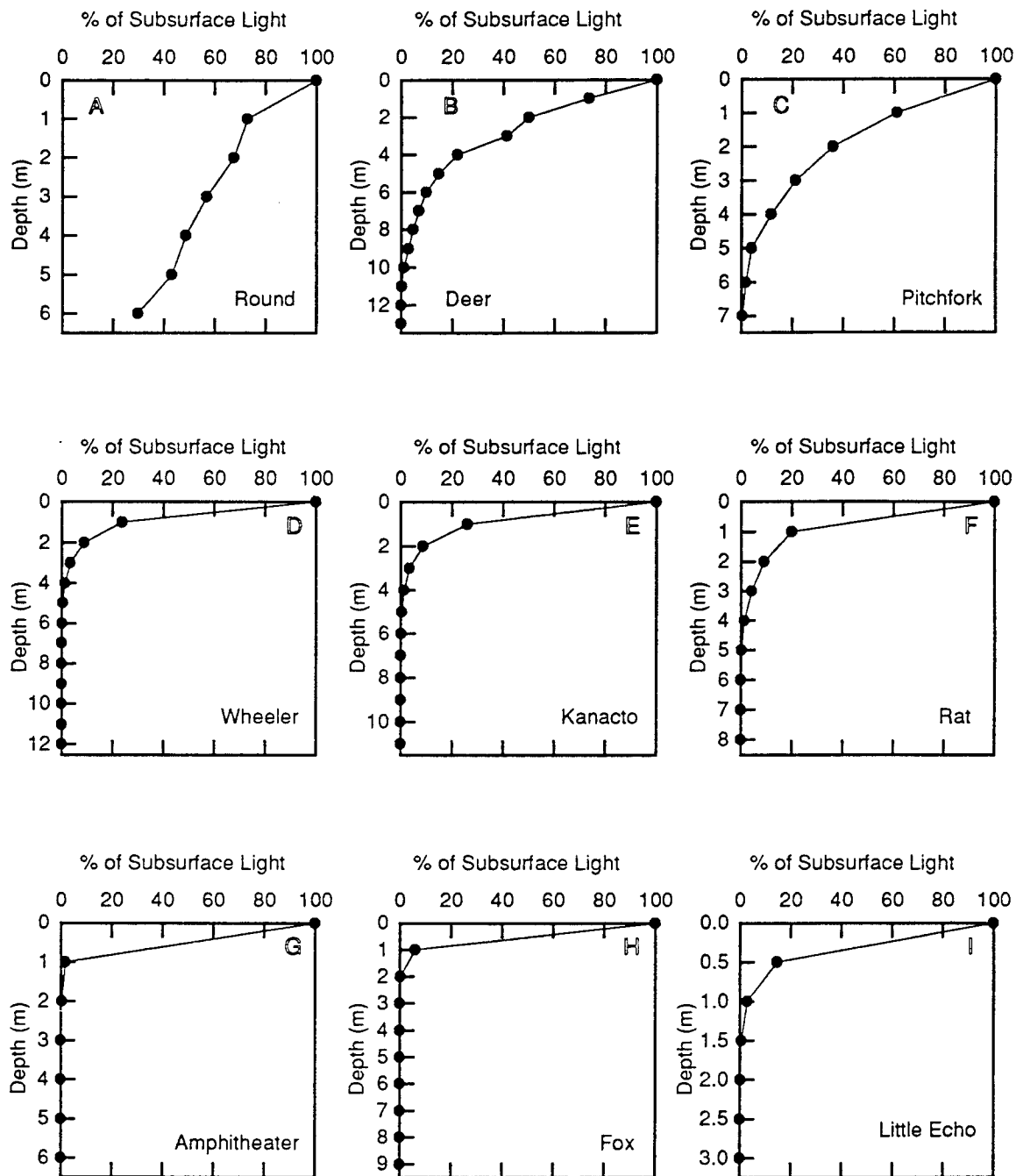
Figure 3.2. Vertical profiles of light in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.

thermocline in all three mid DOC ponds (4 m, Fig. 3.2d-f), but occurred near the top of the thermocline in the high DOC ponds (1 m, Fig. 3.2g-i).

% oxygen saturation

Eight of the nine seepage ponds had little or no oxygen in their hypolimnia (Fig. 3.3). Of these eight sites, four had anoxic hypolimnia. These included two mid DOC ponds (Fig. 3.3e, f) and two high DOC ponds (Fig. 3.3g, h). Oxygen concentrations in the epilimnia of the three high DOC ponds were significantly below saturation (<65%). In Deer Pond, oxygen supersaturation was measured in the mid-metalimnion. Supersaturation at this depth was a consistent feature in this pond throughout the summer during the earlier study by Saunders (1992).

Pigments

Most of the ponds in this study are classified as meso-oligotrophic by the Adirondack Lakes Survey Corporation according to upper water column chlorophyll *a* concentration (Likens, 1975; Fig. 3.4b-i). Round Pond is considered oligotrophic based on the occurrence of low chlorophyll *a* concentrations throughout the water column (<1.3-3.2 μg L^{-1}; Fig. 3.4a). Deep chlorophyll maxima (DCM) were evident in four of the nine seepage ponds. These maxima generally occurred at the base of the metalimnia, and chlorophyll *a* concentrations were considerably greater at these depths than average chlorophyll *a* concentrations in the epilimnia. Concentrations of chlorophyll *a* in the DCM of Pitchfork Pond and Rat Pond were between 27 and 33 μg L^{-1} (Fig. 3.4c, f). In Little Echo Pond, chlorophyll *a* concentration was nearly 50 μg L^{-1} in the DCM (Fig. 3.4i). Chlorophyll *a* concentration in the DCM at Deer Pond approached 70 μg L^{-1} (Fig. 3.4b).
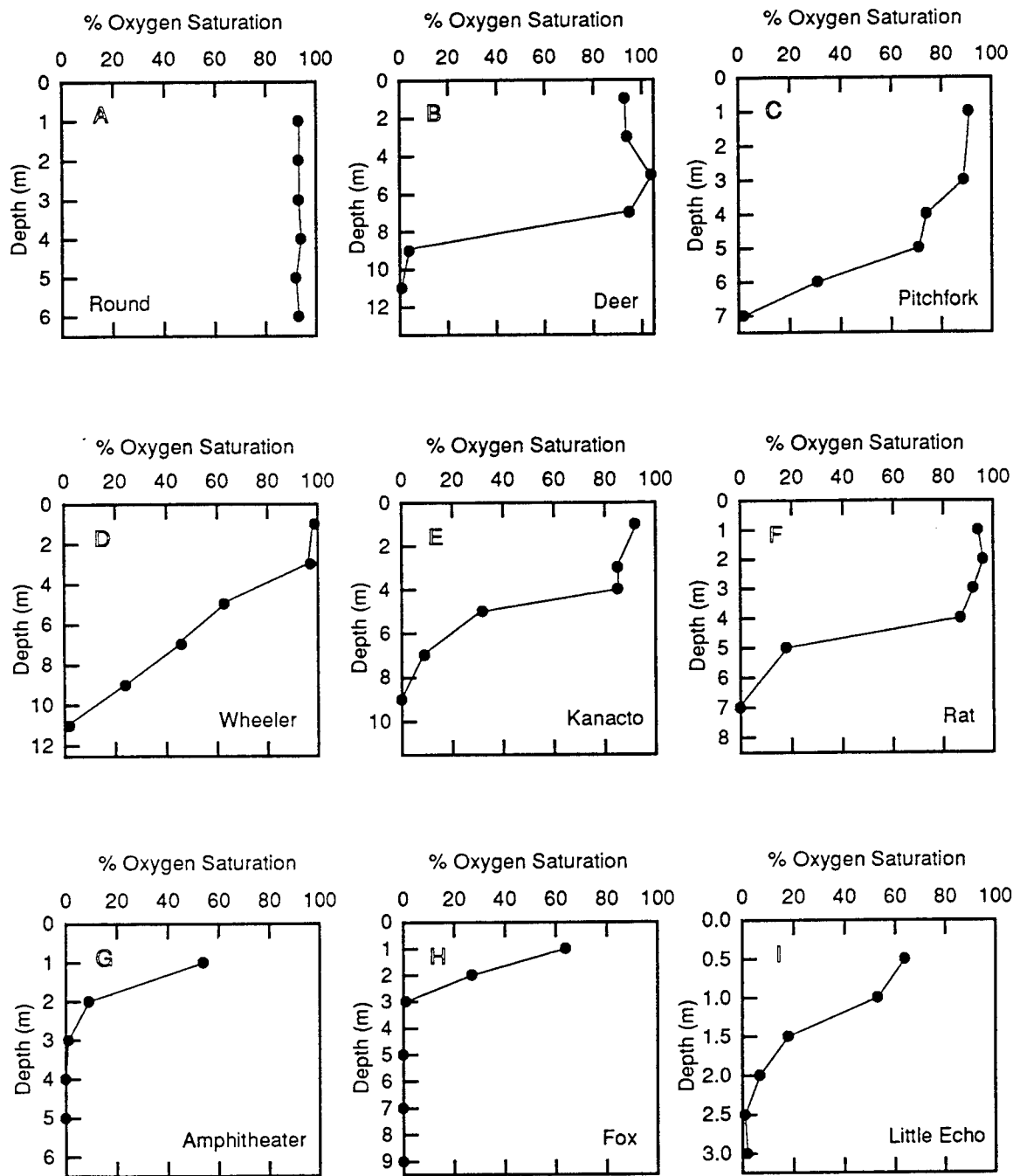
Figure 3.3. Vertical profiles of % oxygen saturation in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.

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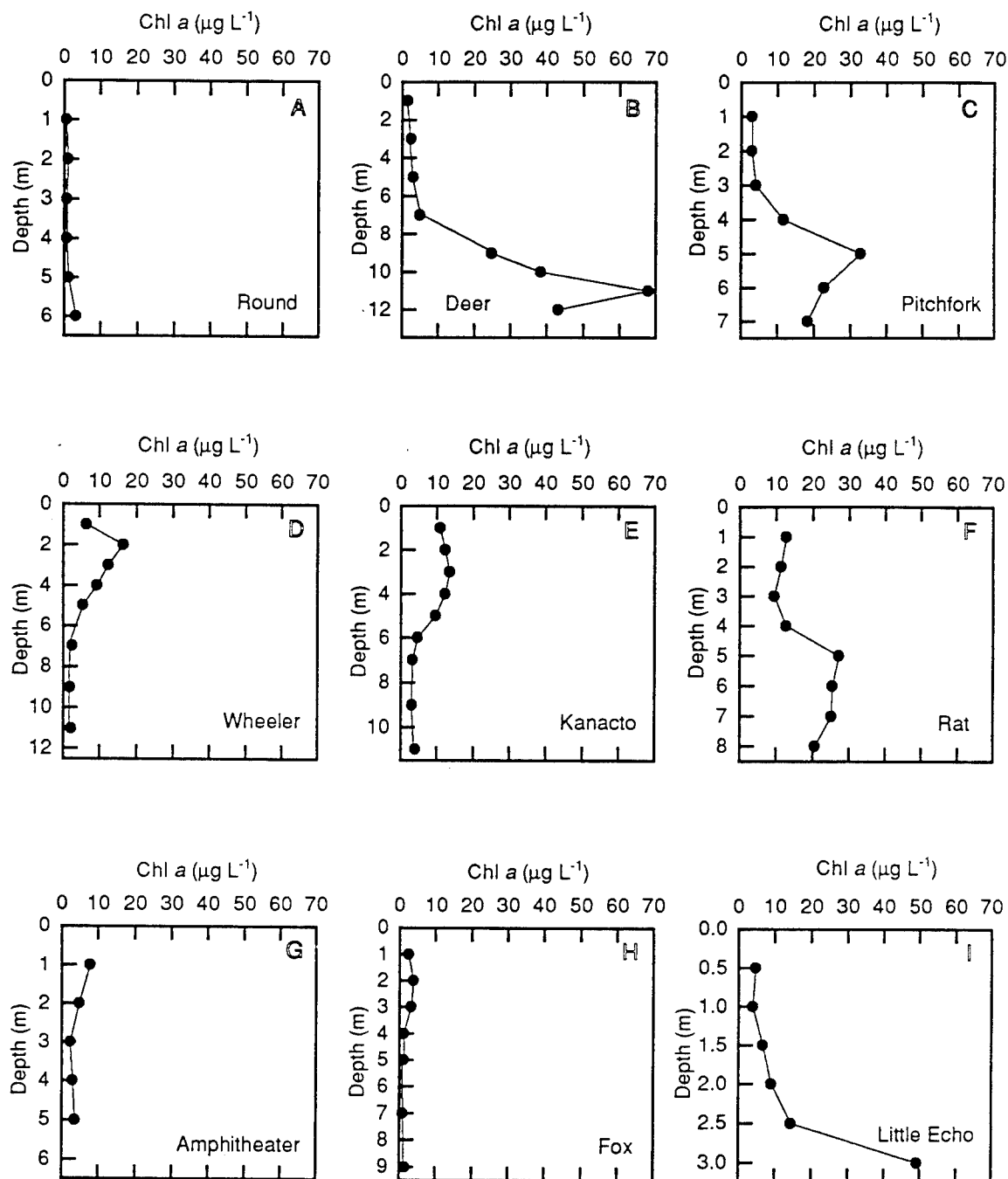
Figure 3.4. Vertical profiles of chlorophyll *a* concentration in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.

Nutrients

Ammonium was the dominant form of dissolved inorganic nitrogen in the epilimnia of all nine seepage ponds and in the hypolimnia of eight of the nine seepage ponds. Epilimnetic NH_4^+ concentrations were positively correlated with DOC concentration (Fig. 3.5a) and ranged from 3.3-16.1 μM NH_4^+ (Table 3.2). NH_4^+ concentrations were generally higher in the hypolimnia (1.7-41.7 μM NH_4^+). Nitrate concentrations in the epilimnia of Wheeler Pond and Fox Pond were 1.3 μM and 1.6 μM, respectively. Epilimnetic NO_3^- was undetectable in the other seven seepage ponds (<0.3 μM). NO_3^- was also undetectable in the hypolimnia of all of the ponds except in the hypolimnion of Wheeler Pond where NO_3^- was the dominant form of inorganic nitrogen (15.7 μM NO_3^- compared to 5.6 μM NH_4^+).

Dissolved inorganic phosphorus (PO_4^{-3}) and total phosphorus (TP) concentrations were generally higher in the hypolimnia than in the epilimnia (Table 3.2). Hypolimnetic PO_4^{-3} concentrations in the nine ponds ranged from 0.10-1.11 μM PO_4^{-3}. TP concentrations in the hypolimnia ranged from 0.13-1.96 μM TP. Epilimnetic PO_4^{-3} and TP concentrations were found to be positively correlated with DOC concentration in the ponds (Fig. 3.5b, c). Epilimnetic PO_4^{-3} and TP concentrations ranged from 0.11-0.23 μM PO_4^{-3} and from 0.13-0.29 μM TP in the low DOC ponds, from 0.23-0.41 μM PO_4^{-3} and 0.37-0.44 μM TP in the mid DOC pond, and from 0.20-0.46 μM PO_4^{-3} and 0.44-0.50 μM TP in the high DOC ponds.

Microbial population abundances

Bacterial abundances (BACT) in the hypolimnia of five of the nine seepage ponds were significantly higher than bacterial abundances in the epilimnia of these ponds, and hypolimnetic abundances ranged from >5×10^6 to ~1.7×10^7 BACT ml^{-1} (Fig. 3.6b, c, f, g).
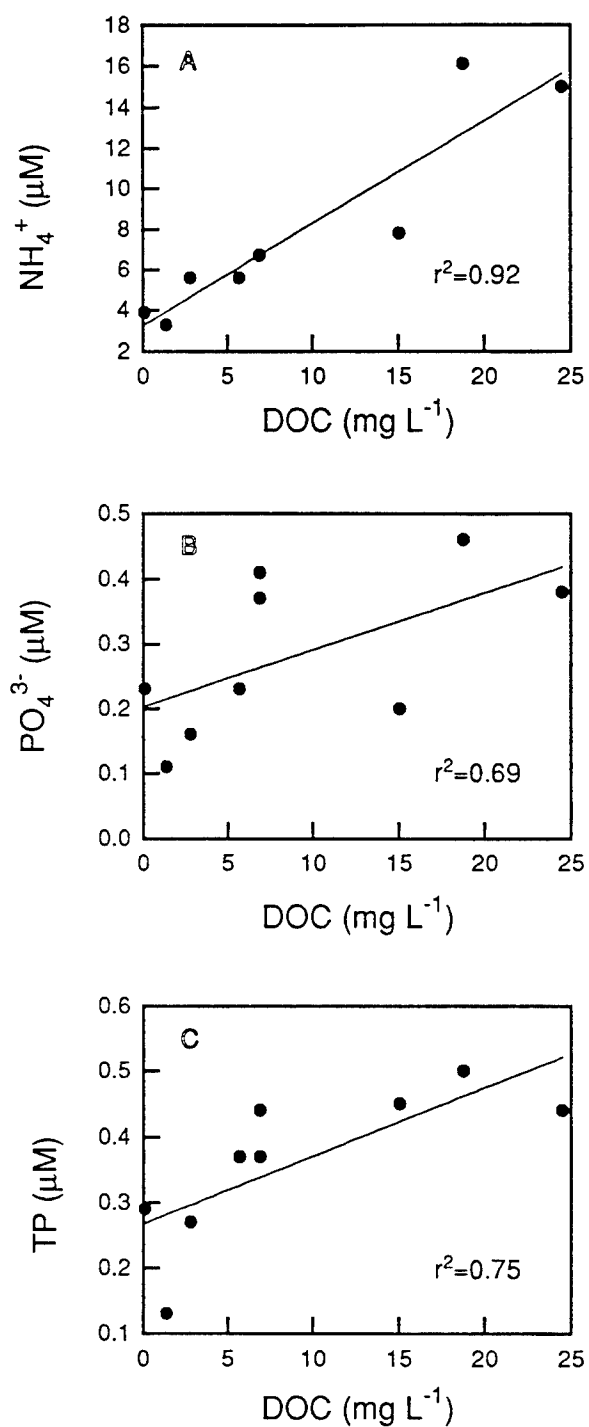
Figure 3.5. The correlation between dissolved organic carbon concentration (DOC) and epilimnetic concentrations of ammonium (A), dissolved phosphate (B) and total phosphorus (C) in nine Adirondack seepage ponds during July 1992.

Table 3.2. Nutrient concentrations during July 1992 in epilimnetic and hypolimnetic water samples collected from low DOC seepage ponds (Round, Deer and Pitchfork), mid DOC seepage ponds (Wheeler, Kanacto and Rat) and high DOC seepage ponds (Amphitheater, Fox and Little Echo) located in the northwest and west central regions of the Adirondack Mountains, NY.

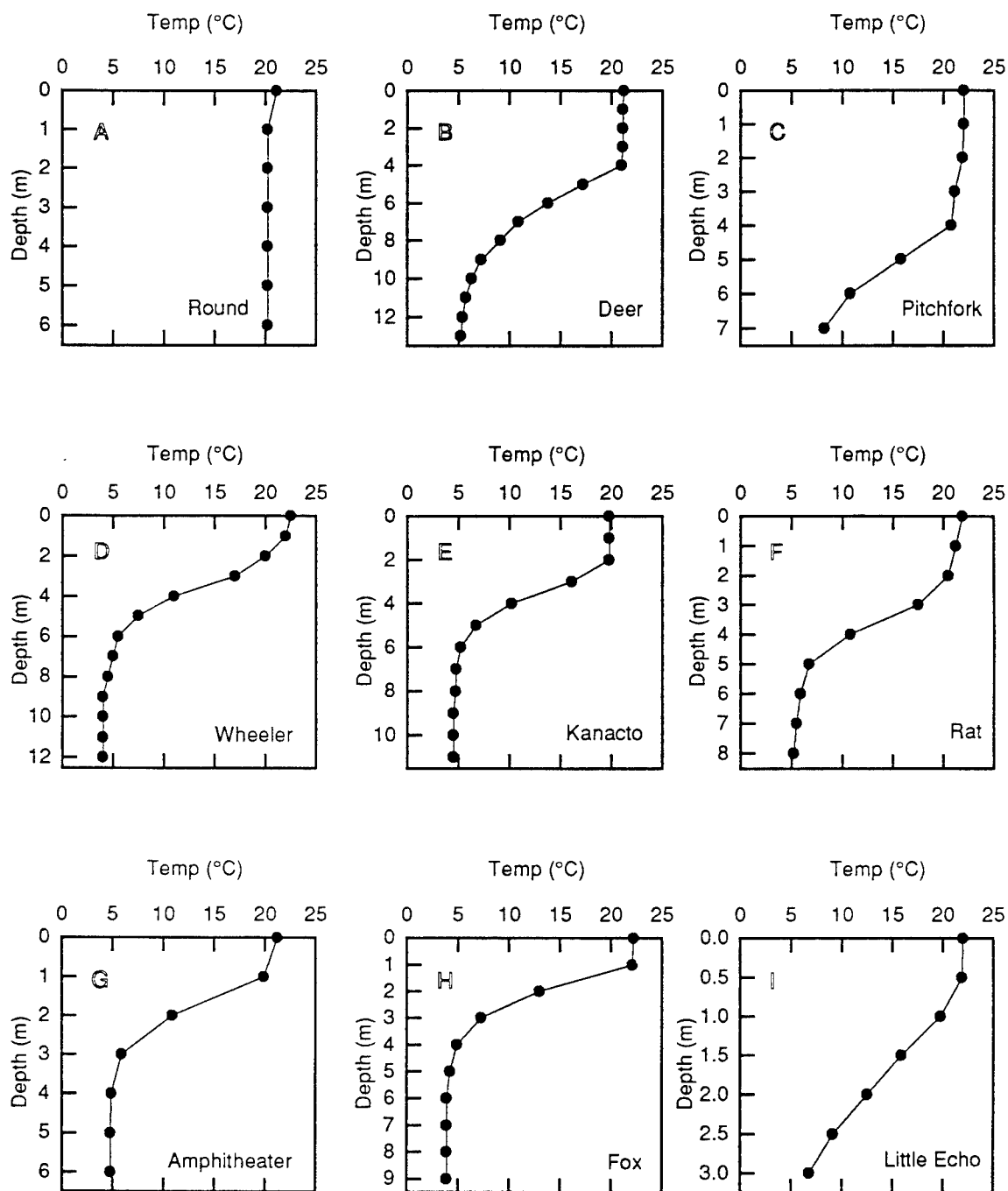| Pond	Date	Depth (m)	NH_4 (µM)	NO_3 (µM)	SRP (PO_4) (µM)	TP (µM)
ROUND	7/16/92	1	3.3	<0.3	0.11	0.13
		5	1.7	<0.3	0.10	0.19
DEER	7/29/92	3	5.6	<0.3	0.16	0.27
		11	30.0	<0.3	0.49	1.50
PITCHFORK	7/20/92	3	3.9	<0.3	0.23	0.29
		7	13.9	<0.3	0.58	1.96
WHEELER	7/19/92	1	5.6	1.3	0.23	0.37
		9	5.6	15.7	0.22	0.32
KANACTO	7/14/92	1	6.7	<0.3	0.37	0.37
		5	35.0	<0.3	0.49	0.60
RAT	7/18/92	1	6.7	<0.3	0.41	0.44
		5	18.9	<0.3	0.44	1.52
AMPHITHEATER	7/22/92	1	15.0	<0.3	0.38	0.44
		3	13.3	<0.3	0.65	0.69
FOX	7/21/92	1	7.8	1.6	0.20	0.45
		7	41.7	<0.3	1.11	1.32
LITTLE ECHO	7/17/92	1	16.1	<0.3	0.46	0.50
		3	25.0	<0.3	1.08	1.74
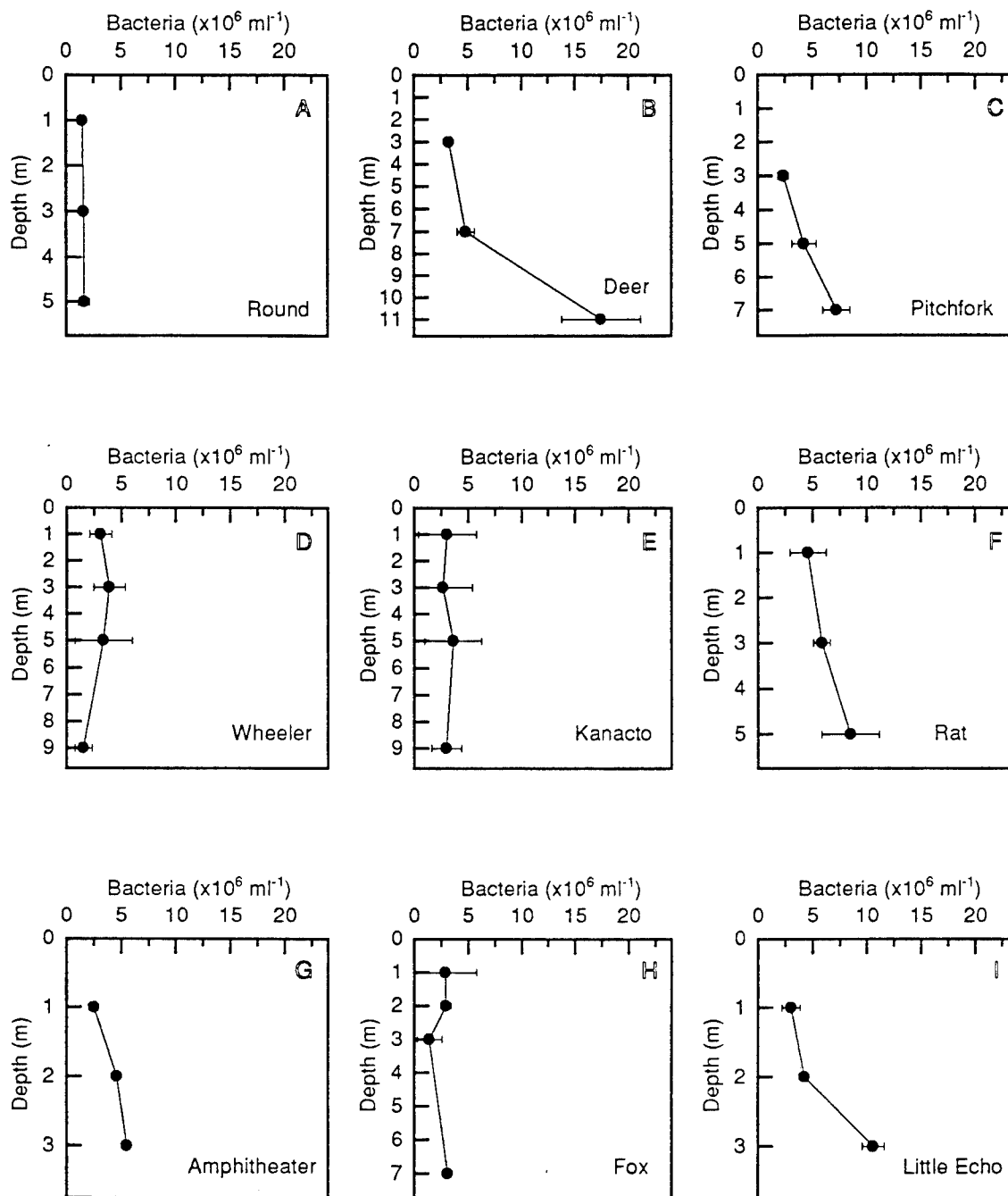
Figure 3.6. Vertical profiles of bacterial abundance (±1 SD) in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.

Bacterial abundances (BACT) remained relatively constant with depth ($\sim 2 \times 10^6$ to $\sim 5 \times 10^6$ BACT ml^{-1}; Fig. 3.7a, d, e, h) in Round Pond, Fox Pond and two mid DOC ponds. Nevertheless, a weak, but significant, correlation existed between bacterial abundance and temperature, % of subsurface light and dissolved oxygen (Fig. 3.7). Bacterial abundance was positively correlated with heterotrophic nanoplankton (HNAN) abundance (Fig 3.8). Upper water column (epilimnion & metalimnion) HNAN abundances in the ponds ranged from $<1 \times 10^4$ to nearly 4×10^4 HNAN ml^{-1}, whereas hypolimnetic HNAN abundances ranged from $<1 \times 10^4$ to $\sim 9 \times 10^4$ HNAN ml^{-1} (Fig. 3.9).

A significant, although weak, negative correlation was observed between phototrophic nanoplankton (PNAN) abundance and DOC concentration in the ponds (Fig. 3.10a). In addition, significant, but weak, negative correlations were found between upper water column PNAN abundance and epilimnetic NH_4^+, PO_4^{-3} and TP concentration (Fig. 3.10b, c, d). The depth distribution of PNAN, however, did not appear to be related to pond DOC concentration. PNAN abundances in Round Pond, which was very clear and well mixed to the bottom, increased with depth (Fig. 3.11a). PNAN abundances ranged from $\sim 1.3 \times 10^4$ ml^{-1} in the epilimnion to 2.3×10^4 ml^{-1} in the hypolimnion. Subsurface peaks in PNAN abundance that ranged from $<2 \times 10^4$ to $>4 \times 10^4$ PNAN ml^{-1} occurred in Deer, Pitchfork, Wheeler, Fox and Little Echo Ponds (Fig. 3.11b-d, h, i). PNAN abundances did not change greatly with depth in Rat Pond ($\sim 1.7 \times 10^4$ PNAN ml^{-1}; Fig. 3.11f) or Amphitheater Pond (0.5×10^4 PNAN ml^{-1}; Fig. 3.11g). PNAN abundances were also relatively constant in the upper water column of Kanacto Pond ($\sim 2 \times 104$ PNAN ml-1), but decreased significantly in the hypolimnion ($<0.5 \times 10^4$ PNAN ml^{-1}; Fig. 3.11e).

Both solitary and colonial forms of unicellular algae were observed in most ponds. All solitary algae were considered as a single group (solitary PNAN) during this study. No attempt was made to distinguish individual species or genera of solitary algae due to difficulties in identifying cellular features using epifluorescence microscopy. The
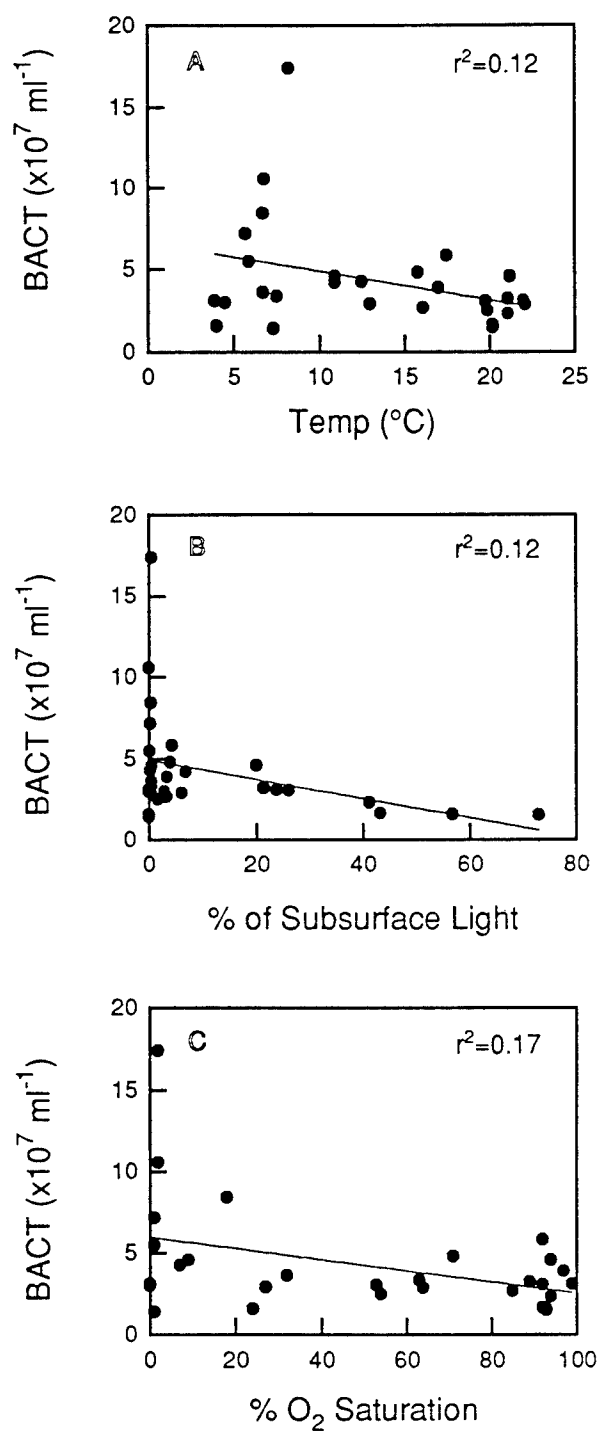
Figure 3.7. Correlations between bacterial abundance (BACT) and temperature (A), % of subsurface light (B), and % oxygen saturation (C) in nine Adirondack seepage ponds during July 1992.

$r^2=0.50$
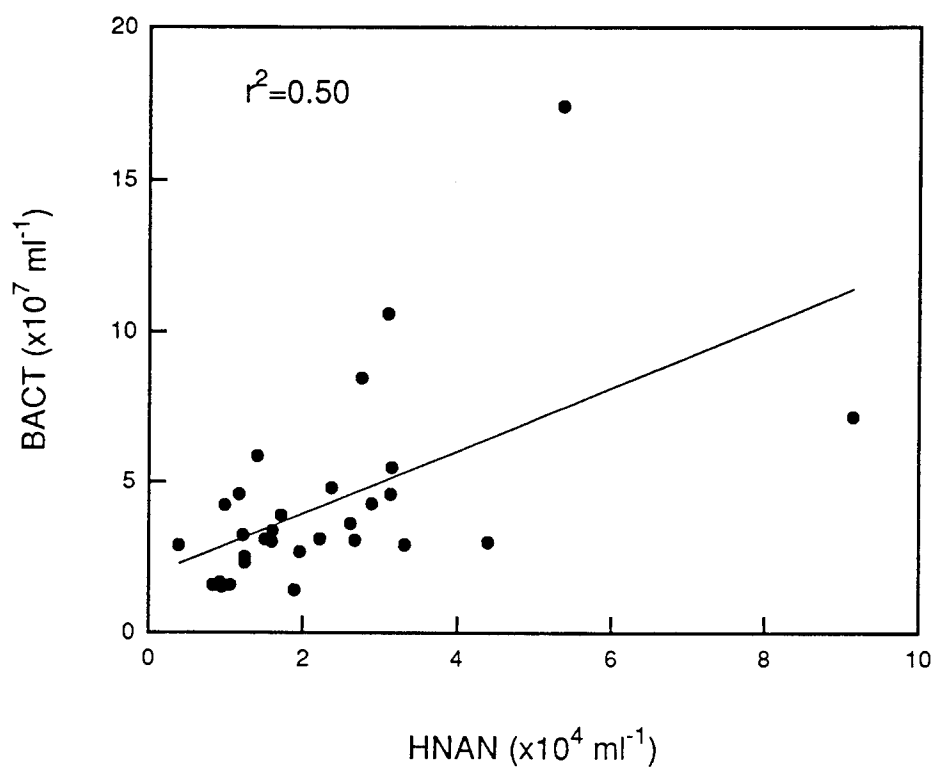
Figure 3.8. The correlation between bacterial abundance (BACT) and heterotrophic nanoplankton abundance (HNAN) for nine Adirondack seepage ponds during July 1992.
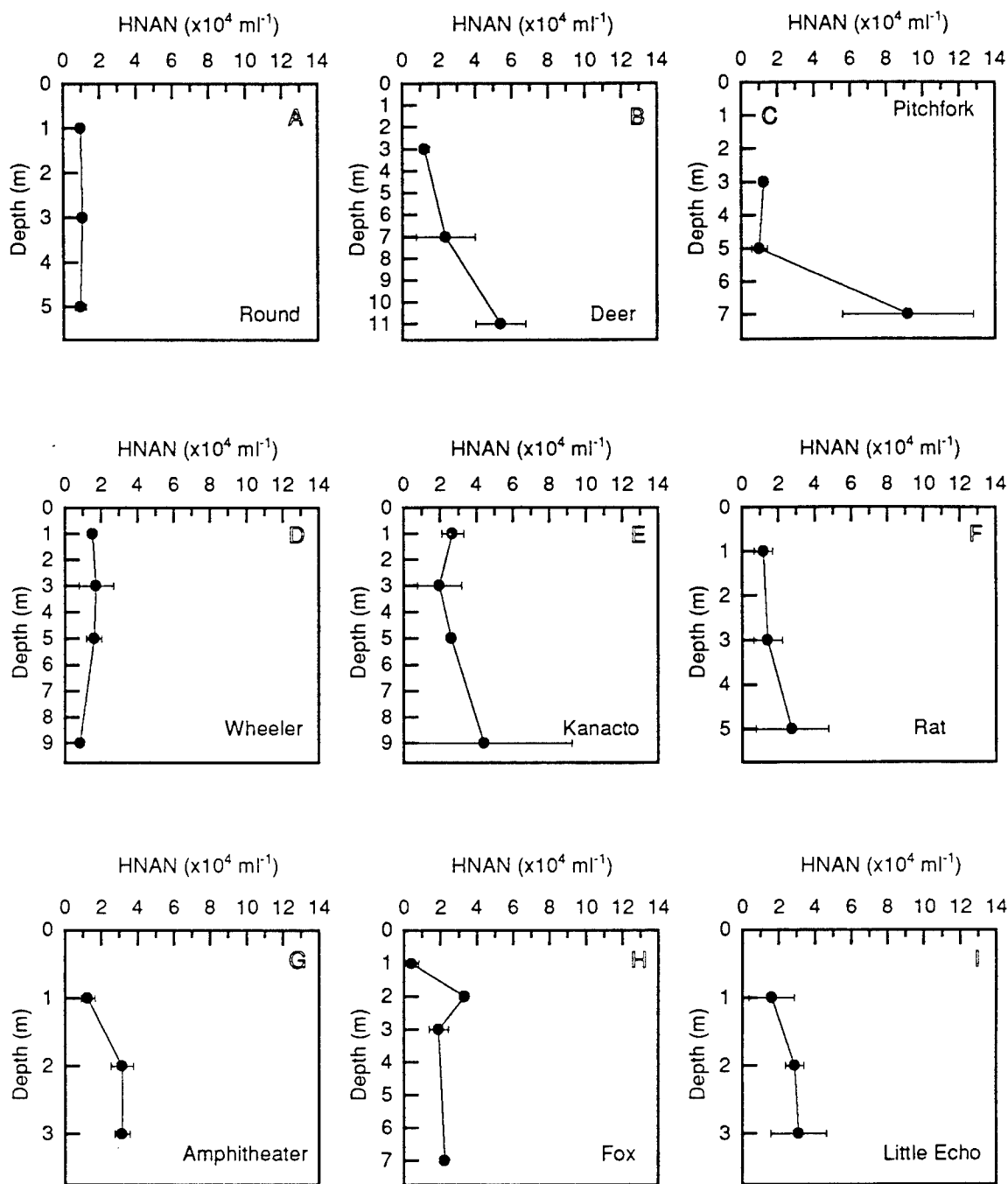
Figure 3.9. Vertical profiles of heterotrophic nanoplankton (HNAN) abundances
(±1 SD) in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the
northwest and west central regions of the Adirondack Mountains, NY during July 1992.

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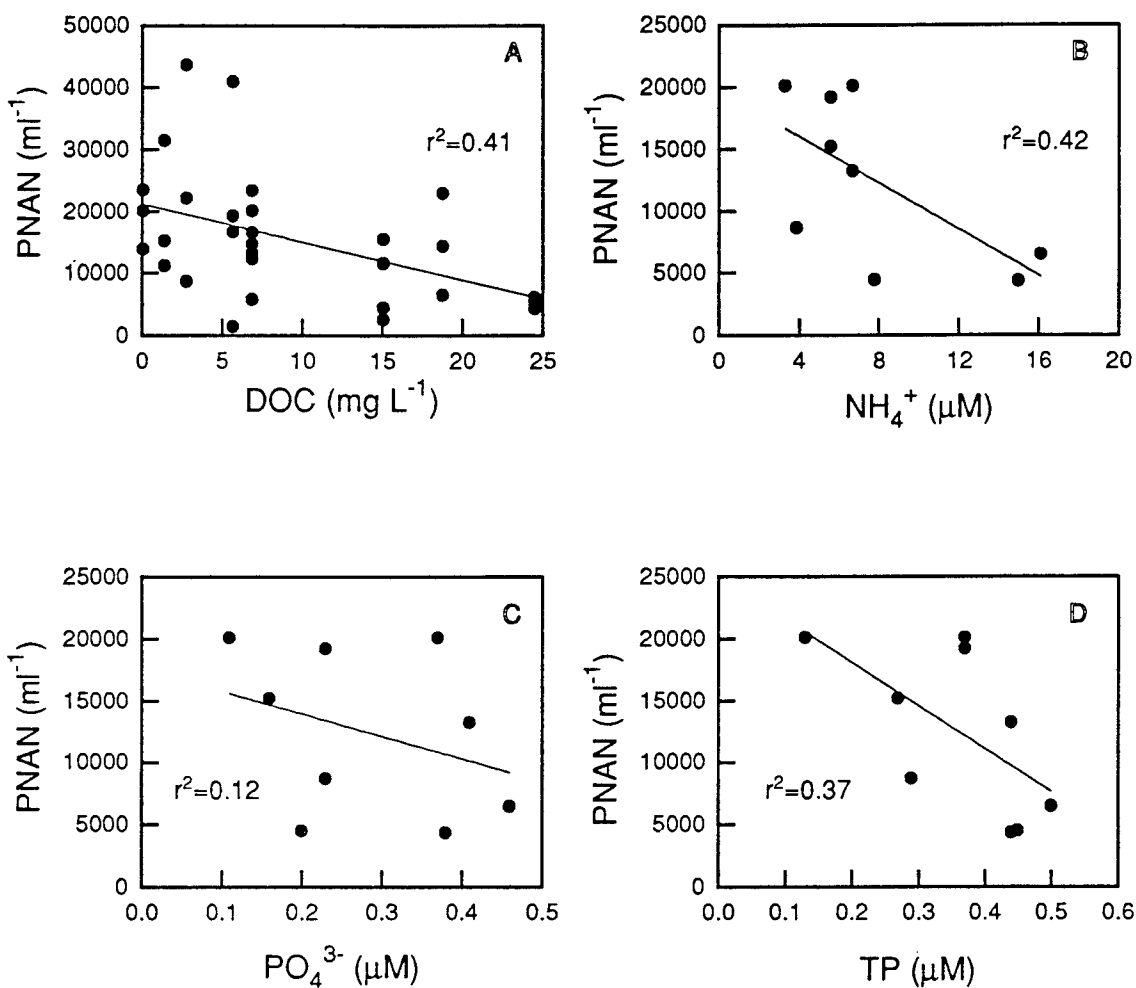
Figure 3.10. Correlations between epilimnetic, metalimnetic and hypolimnetic phototrophic nanoplankton abundance and DOC concentration (A), and between epilimnetic phototrophic nanoplankton abundance and epilimnetic ammonium (B), dissolved phosphate (C) and total phosphorus (D) concentrations in nine Adirondack seepage ponds during July 1992.
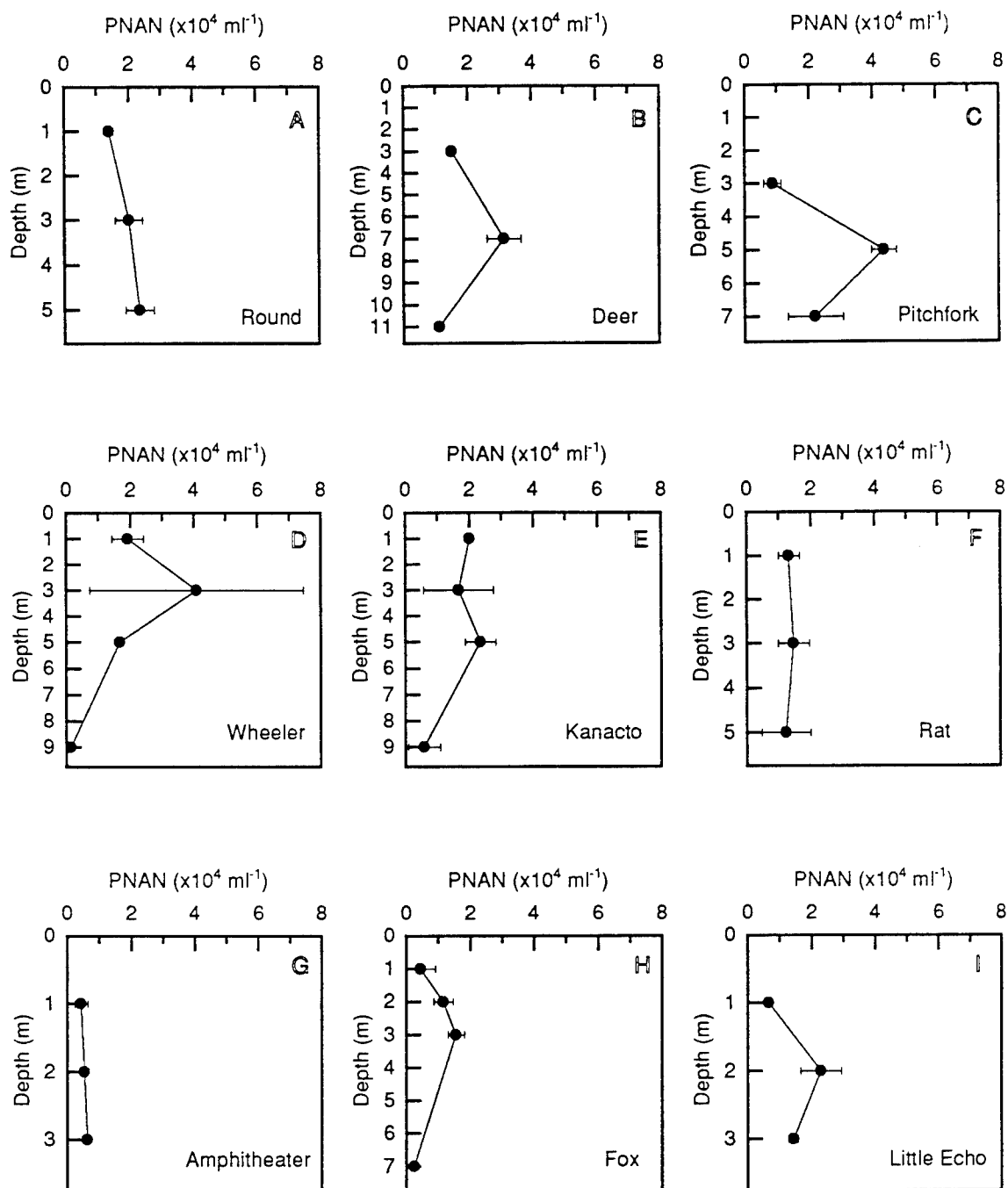
Figure 3.11. Vertical profiles of phototrophic nanoplankton (PNAN) abundance
(±1 SD) in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the
northwest and west central regions of the Adirondack Mountains, NY during July 1992.

colonial chrysophyte *Dinobryon* was clearly identified by the presence of its distinct lorica, however. *Dinobryon*, a known mixotroph, was therefore counted separately from other colonial algae (Colonial PNAN). Solitary algal abundances ranged from $<0.5 \times 10^4$ ml^{-1} to nearly 3×10^4 ml^{-1} (Fig. 3.12), and dominated the phototrophic nanoplankton ($>50\%$) in the epilimnion, metalimnion and hypolimnion in seven of the nine seepage ponds. Solitary forms were the principal component of most of the subsurface PNAN peaks that were observed in some of the ponds (Fig. 3.12b, h, i), but colonial forms of algae dominated the subsurface PNAN peaks in two of the ponds. Greater than 70% of the metalimnetic PNAN populations in Pitchfork Pond and Wheeler Pond were colonial algae. Unidentified colonial algae dominated ($\sim 3 \times 10^4$ ml^{-1}) in the metalimnion of Pitchfork Pond (Fig. 3.12c), but the mixotrophic colonial chrysophyte *Dinobryon* was the largest overall contributor (42% of PNAN abundance) to the PNAN assemblage in the mid-metalimnion of Wheeler Pond and abundances were high ($\sim 1.7 \times 10^4$ *Dinobryon* ml^{-1}; Fig. 3.12d).

Mixotrophic nanoplankton were numerically important in the upper water column of Wheeler Pond accounting for $\sim 25\%$ of the total nanoplanktonic algal assemblage (Fig. 3.13d). Absolute abundances of mixotrophic algae were highest in the metalimnion of this pond ($\sim 2 \times 10^4$ ml^{-1}), and the most important nanoplanktonic mixotroph at this depth was *Dinobryon* (85% of MNAN), although only $\sim 50\%$ of the *Dinobryon* populations in both the epilimnion ($\sim 1 \times 10^3$ ml^{-1}) and metalimnion ($>9 \times 10^3$ ml^{-1}) were phagotrophically active (Fig. 3.14d). In addition, mixotrophic nanoplankton were moderately important (15-20% of total PNAN) in the epilimnia of Amphitheater Pond and Little Echo Pond (Fig. 3.13g, i), but absolute abundances were modest relative to abundances in the metalimnion of Wheeler Pond. *Dinobryon* and solitary forms of mixotrophic nanoplankton were both present at abundances of ~ 500 ml^{-1} in the epilimnion of Amphitheater Pond (Fig. 3.14g), while solitary forms dominated the mixotrophic nanoplankton in the epilimnion of Little Echo Pond (~ 750 ml^{-1}; Fig. 3.14i). Mixotrophic
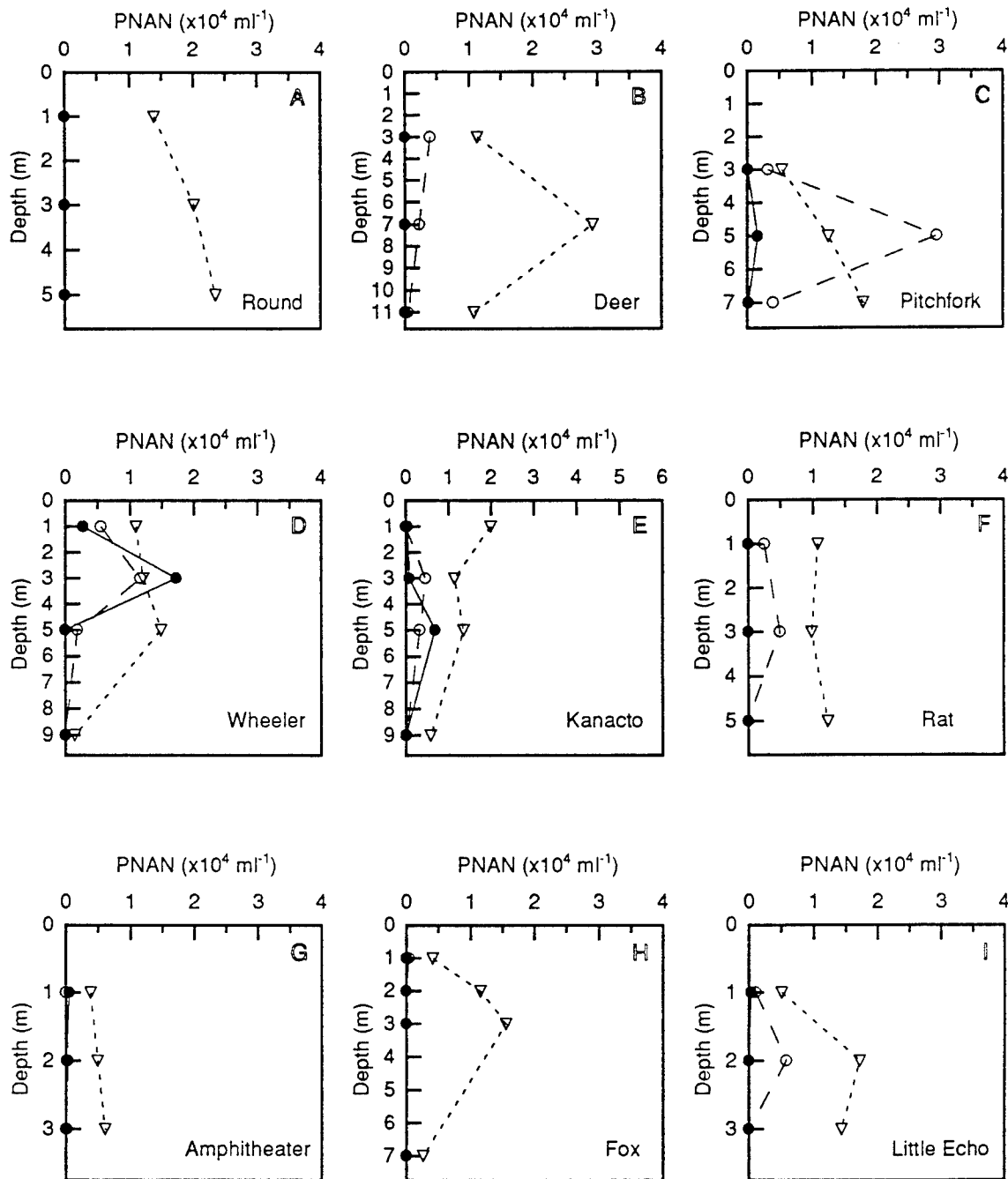
Figure 3.12. Vertical profiles of unidentified solitary phototrophic nanoplankton (▽), colonial phototrophic nanoplankton (○) and *Dinobryon* (●) abundances (±1 SD) in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.
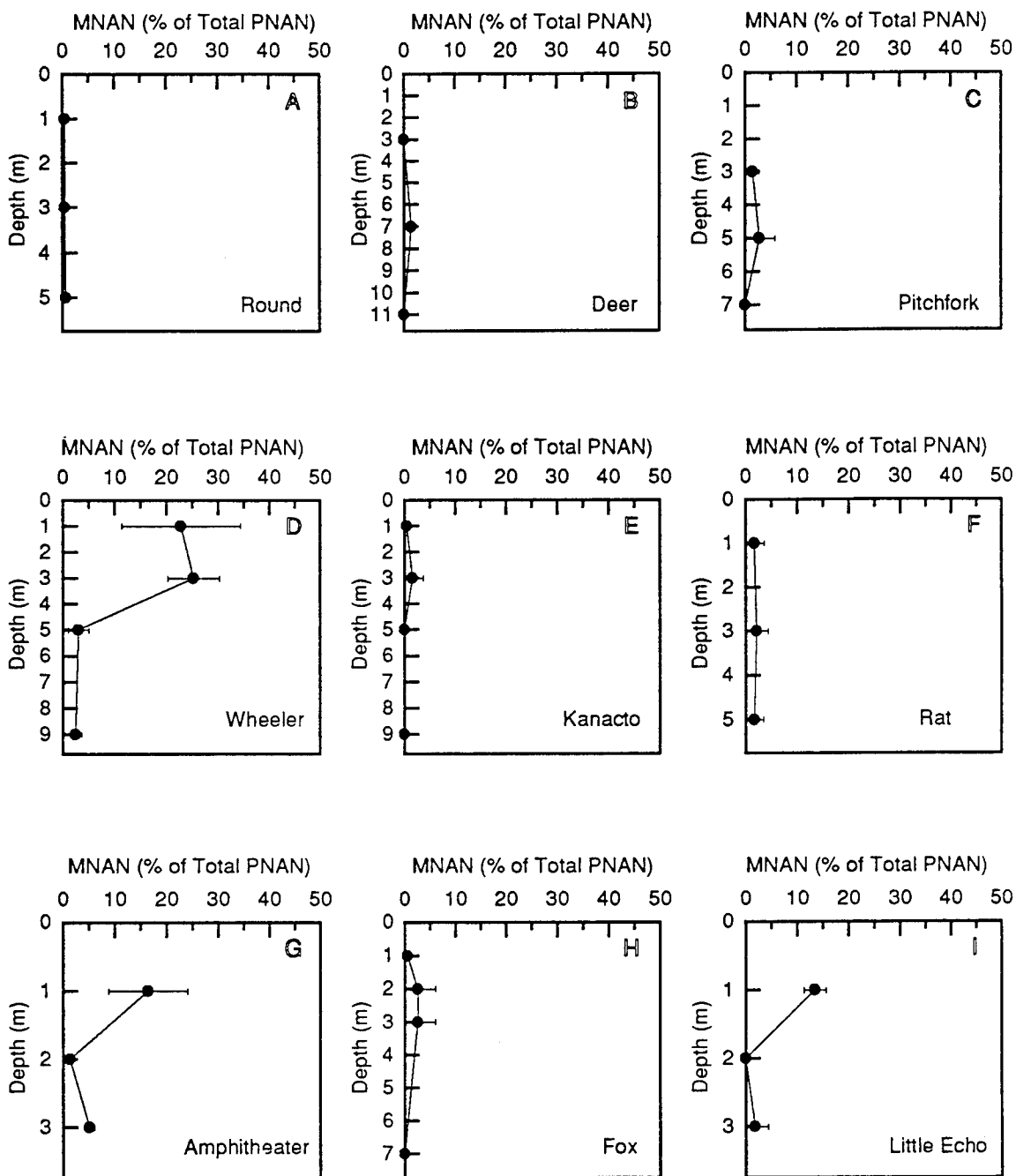
Figure 3.13. Vertical profiles of the percent (±1 SD) of the nanoplanktonic phototroph assemblage that were mixotrophic in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.
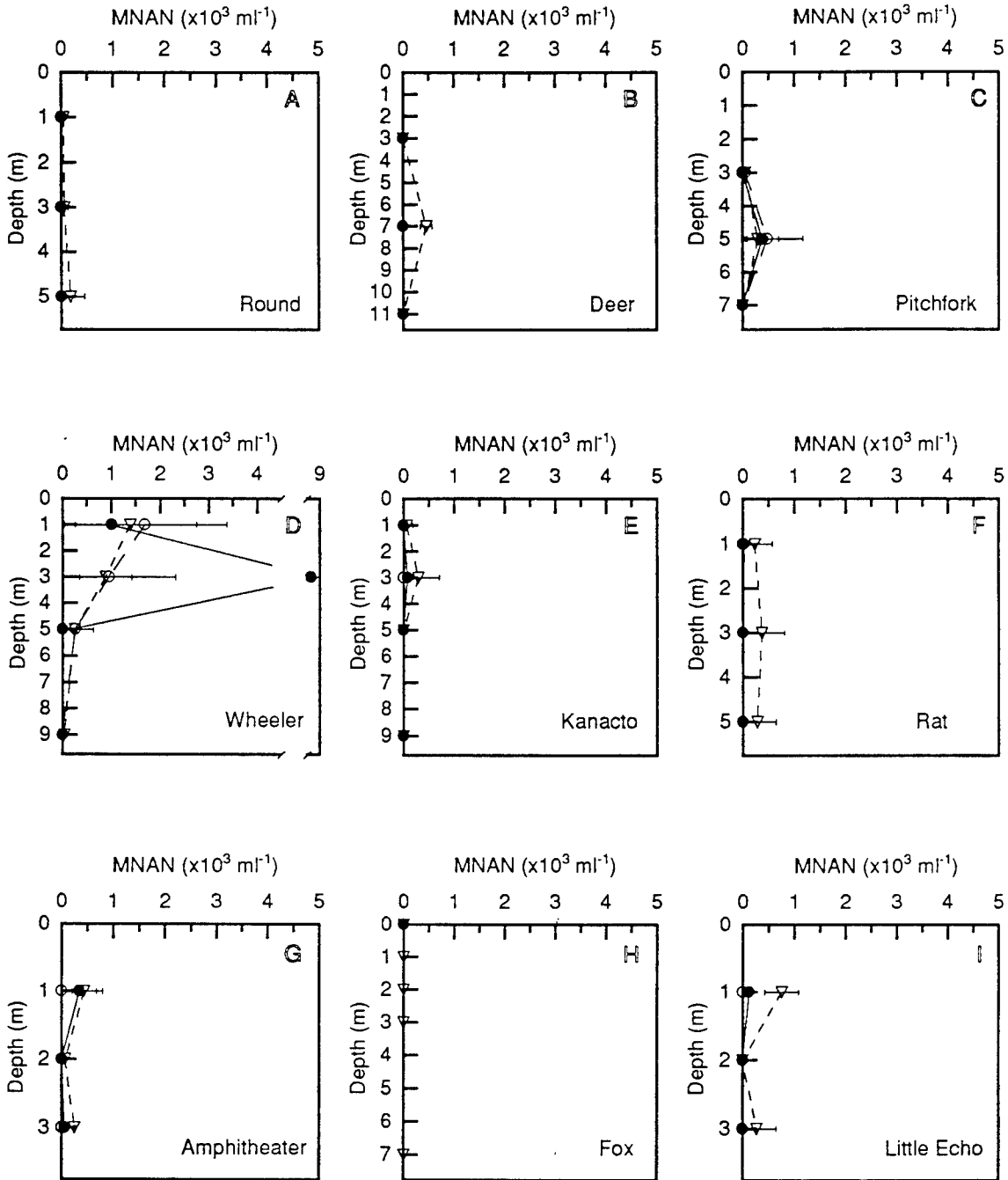
Figure 3.14. Vertical profiles of abundances (±1 SD) of unidentified solitary mixotrophic nanoplankton (∇), unidentified colonial mixotrophic nanoplankton (○) and phagotrophically active *Dinobryon* (●) in low DOC (A-C), mid DOC (D-F) and high DOC (G-I) seepage ponds in the northwest and west central regions of the Adirondack Mountains, NY during July 1992.

nanoplankton comprised less than 5% of the total PNAN assemblages in the remaining ponds (Fig. 3.13a-c, e, f, h).

Coastal Salt Ponds

The coastal salt ponds examined in this study reside in an outwash plain formed during glacial recession. These small, semi-enclosed coastal lagoons remain connected to the ocean and exhibit small tidal fluctuations. The extent of tidal exchange in the ponds is dependent on the size of the connecting passage to adjacent coastal waters, basin morphology and depth (See Table. 3.1 for surface areas and basin depths) and the degree of freshwater influx. Oyster Pond and Salt Pond have relatively deep main basin depths (4-6 m) compared with the main basin of Perch Pond (<3 m). Both Oyster Pond and Salt Pond are also connected directly through narrow culverts to Vineyard Sound, while Perch Pond is indirectly connected to Vineyard Sound by a shallow connection (sill depth <0.5 m) to a large embayment that empties into Vineyard Sound. All three ponds have small riverine inputs near the head of the ponds.

Physical characteristics

Water column stratification differed markedly between the three salt ponds (Fig. 3.15). Perch Pond was fairly well mixed as a result of wind-driven circulation in the shallow basin. A shallow lens (~0.5 m thick) of slightly cooler (24°C), less saline water (~17 ‰) rested over a relatively well mixed bottom layer of warmer (26.5°C), 24 ‰ water (Fig. 3.15a). The 4 m basin in Oyster Pond was weakly stratified. The surface layer was well-mixed and was characterized by warm (26.5°C), brackish (~7 ‰) water that extended down to a depth of 2 m. Below this depth, water temperature gradually decreased as salinity increased. Water temperature was ~18°C and salinity was

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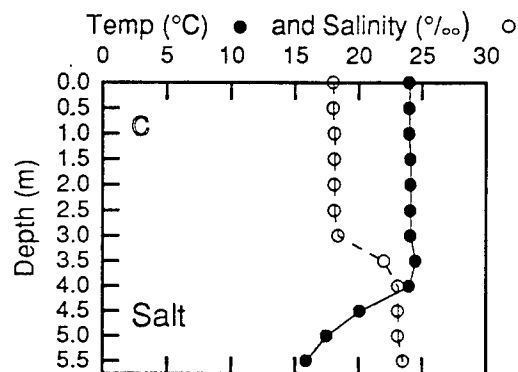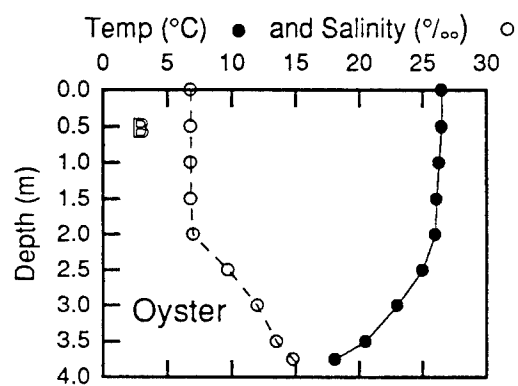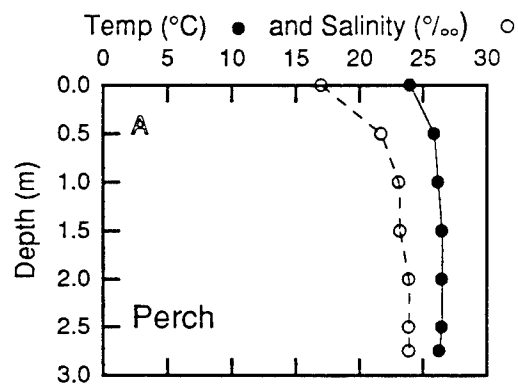
Figure 3.15. Temperature and salinity profiles for Perch Pond (A), Oyster Pond (B) and Salt Pond (C), Falmouth, MA determined during early summer 1993.

approximately 15 ‰ near the bottom (Fig. 3.15b). Salt Pond was well-stratified at the time of sampling. The surface mixed layer was relatively deep and extended down to 3 m. A sharp halocline was present between 3 and 4 m where salinity increased from 18 ‰ to nearly 23 ‰. Temperature decreased significantly below 4 m to ~16°C near the bottom (Fig. 3.15c).

Light attenuation rates appeared to be similar in the three coastal ponds (Fig. 3.16), although the availability of light in the bottom layers was less in ponds with deeper basin depths. Greater than 1% of subsurface light was available throughout the water column in Perch Pond (Fig. 3.16a). One percent of subsurface light was reached near the bottom of Oyster Pond at a depth of 3.5 m (Fig. 3.16b). The depth of 1% of subsurface light in Salt Pond was 4 m. This depth coincided with the top of the thermocline in Salt Pond (Fig. 3.16c).

% oxygen saturation

Oxygen supersaturation was evident in the upper layer of Perch Pond and Oyster Pond (Fig. 3.17a, b). O_2 saturation decreased from ~105% to ~90% between 0.5 m and 1 m in Perch Pond, but the water column remained saturated with oxygen to a depth of 3 m in Oyster Pond. O_2 concentrations in Oyster Pond decreased rapidly from ~100% saturation to ~10% saturation between 3 m and 3.5 m. Oxygen concentrations near the surface of Salt Pond were <95% saturated and decreased gradually to ~80% saturation at a depth of ~3 m (Fig. 3.17c). O_2 saturation levels decreased rapidly in the halocline of Salt Pond and became stable at ~10% saturation in the bottom layer. None of the three ponds exhibited bottom water anoxia.

Figure 3.16. Vertical profiles of light iin Perch Pond (A), Oyster Pond (B) and Salt Pond (C), Falmouth, MA determined during early summer 1993.
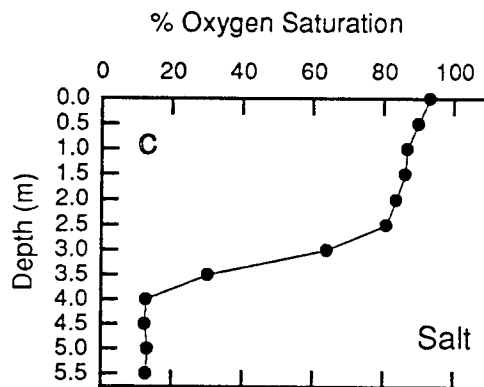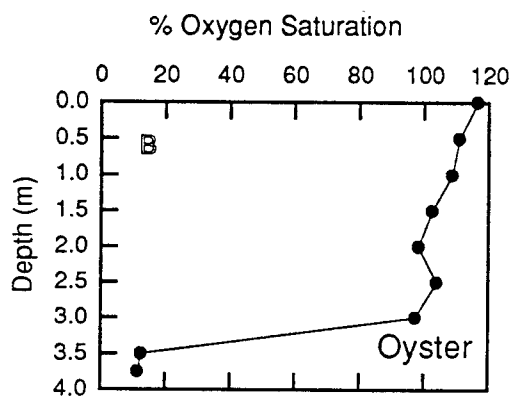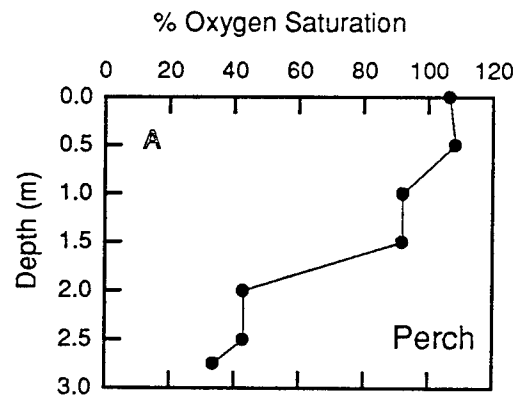
Figure 3.17. Vertical profiles of % oxygen saturation in Perch Pond (A), Oyster Pond (B) and Salt Pond (C), Falmouth, MA determined during early summer 1993.

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Pigments

Upper water column chlorophyll *a* and carotenoid concentrations in Perch Pond were nearly an order of magnitude higher than upper water column concentrations in Salt Pond (Fig. 3.18). Water samples collected for chlorophyll analyses from Oyster Pond were mishandled. Perch Pond chlorophyll *a* concentrations were ~30 µg L^{-1} in the upper water column (0-1m; Fig. 3.18a). A peak in chlorophyll *a* concentration (~70 µg L^{-1}) was observed at a depth of 2 m. Carotenoid concentrations were lower than chlorophyll *a* concentrations in Perch Pond, but mirrored chlorophyll *a* concentrations throughout the water column (Fig. 3.18a). Chlorophyll *a* and carotenoid concentrations mirrored each other in the upper water column of Salt Pond as well. Their concentrations remained relatively constant to a depth of 3.5 m (<4 µg Chl *a* L^{-1}, ~2 µg carotenoid L^{-1}), but below the thermocline both carotenoid and chlorophyll *a* concentrations increased dramatically, and carotenoid concentrations exceeded chlorophyll *a* concentrations by more than 4 fold at this depth (~60 µg Chl *a* L^{-1}, >240 µg carotenoid L^{-1}; Fig. 3.18b).

Nutrients

Ammonium was the dominant form of inorganic nitrogen at each site, except in the surface waters of Perch Pond (Fig. 3.19). Nitrate concentrations (8.8 µM NO$_3^-$) exceeded NH$_4^+$ concentrations (1.8 µM NH$_4^+$) by nearly five fold in the low salinity lens at the surface of Perch Pond, but NO$_3^-$ concentrations decreased rapidly in the top meter from 8.8 µM to <1 µM NO$_3^-$ (Fig. 3.19a). NH$_4^+$ concentrations in Perch Pond remained constant in the top meter and then gradually increased with depth (5 µM NH$_4^+$ at 2.5 m). NH$_4^+$ concentrations in the upper water columns of Oyster Pond and Perch Pond were also on the order of 2 µM NH$_4^+$ and remained relatively constant with depth down to at least the thermocline. NH$_4^+$ concentrations increased dramatically in the thermocline
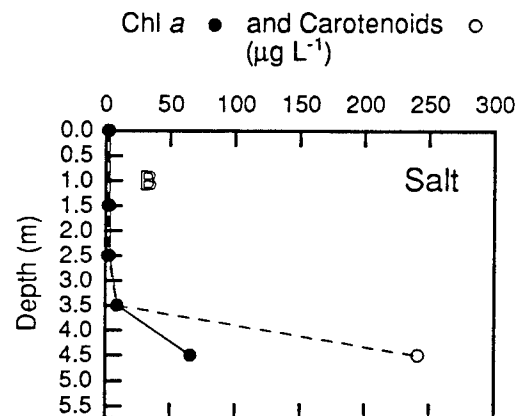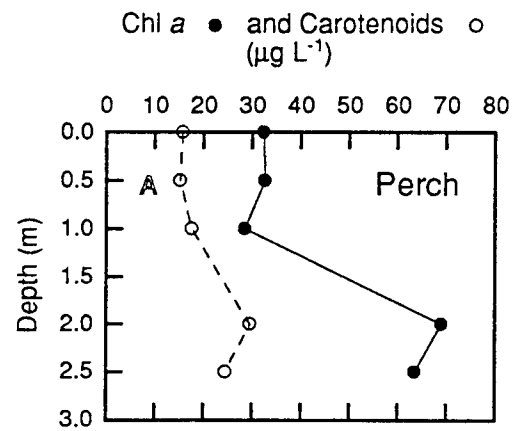
Figure 3.18. Vertical profiles of chlorophyll *a* and carotenoid concentration in Perch Pond (A) and Salt Pond (B), Falmouth, MA determined during early summer 1993.
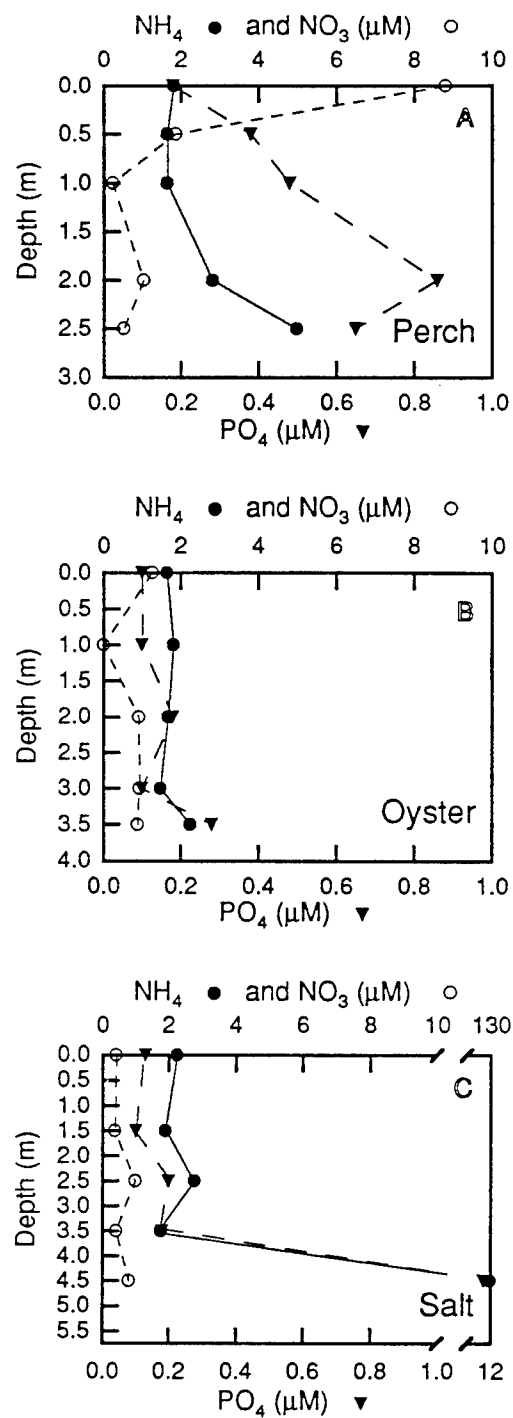
Figure 3.19. Vertical profiles of inorganic nitrogen and phosphorus concentrations in Perch Pond (A), Oyster Pond (B) and Salt Pond (C), Falmouth, MA determined during early summer 1993.

(130 μM NH_4^+) of Salt Pond (Fig. 3.19c), but did not increase significantly (2.25 μM NH_4^+) in the thermocline of Oyster Pond (Fig. 3.19b). NO_3^- concentrations in Oyster Pond and Salt Pond did not vary greatly with depth (0.5-1 μM NO_3^-), although there was a NO_3^- minimum (<0.005 μM NO_3^-) observed at 1 m in Oyster Pond.

Dissolved phosphate tended to mirror NH_4^+ concentrations, particularly in Oyster Pond and Salt Pond. PO_4^{-3} concentrations were generally an order of magnitude lower than NH_4^+ concentrations, and were less than 0.18 μM in surface waters of all three coastal ponds during this study. PO_4^{-3} concentrations in Perch Pond increased steadily from the surface down to 2 m (0.86 μM), unlike NH_4^+ concentrations, and then decreased again below this depth (0.65 μM PO_4^{-3}; Fig. 3.19a). PO_4^{-3} concentrations ranged from 0.10-0.28 μM in Oyster Pond (Fig. 3.20b) and from 0.10-0.20 μM PO_4^{-3} down to the thermocline in Salt Pond. The concentration of PO_4^{-3} below the thermocline in Salt Pond was 11.72 μM (Fig. 3.19c).

Microbial population abundances

Bacterial population abundances were high at all three coastal pond sites ranging from 7×10^6 to 3×10^7 BACT ml^{-1}. In Perch Pond bacterial abundances in surface waters were approximately 1×10^7 ml^{-1} and increased with depth ($\sim 2.5 \times 10^7$ ml^{-1} at 2.5 m; Fig. 3.20a). Bacterial abundances in Oyster Pond did not vary greatly with depth and ranged from $\sim 7 \times 10^6$ to $< 1 \times 10^7$ BACT ml^{-1} (Fig. 3.20d). Bacterial abundances remained relatively constant from the surface to a depth of 3.5 m in Salt Pond ($\sim 1 \times 10^7$ ml^{-1}), and then increased dramatically below this depth to nearly 3×10^7 ml^{-1} (Fig. 3.20g).

Heterotrophic nanoplankton abundances in the low-salinity lens at the surface of Perch Pond were $\sim 1 \times 10^3$ HNAN ml^{-1} and increased to $\sim 1.3 \times 10^4$ HNAN ml^{-1} below 0.5 m (Fig. 3.20b). Heterotrophic nanoplankton abundances in Oyster Pond ranged from $\sim 8 \times 10^3$
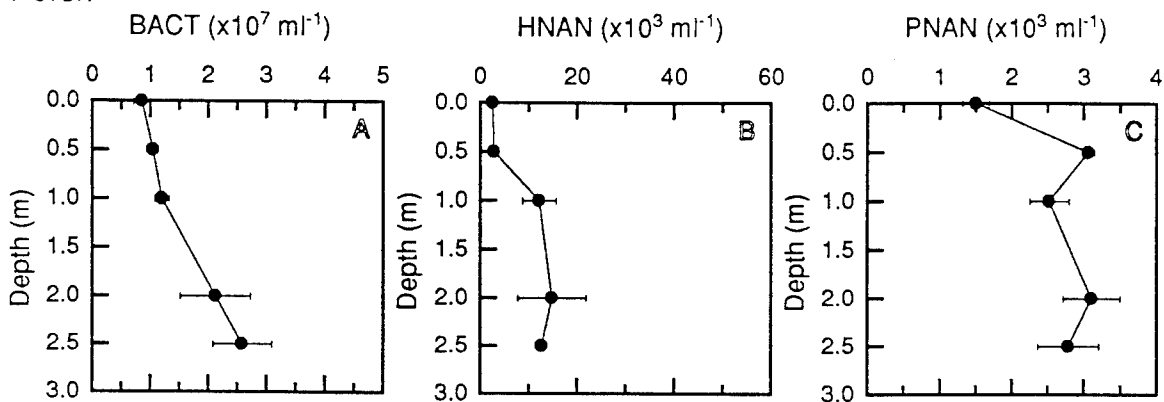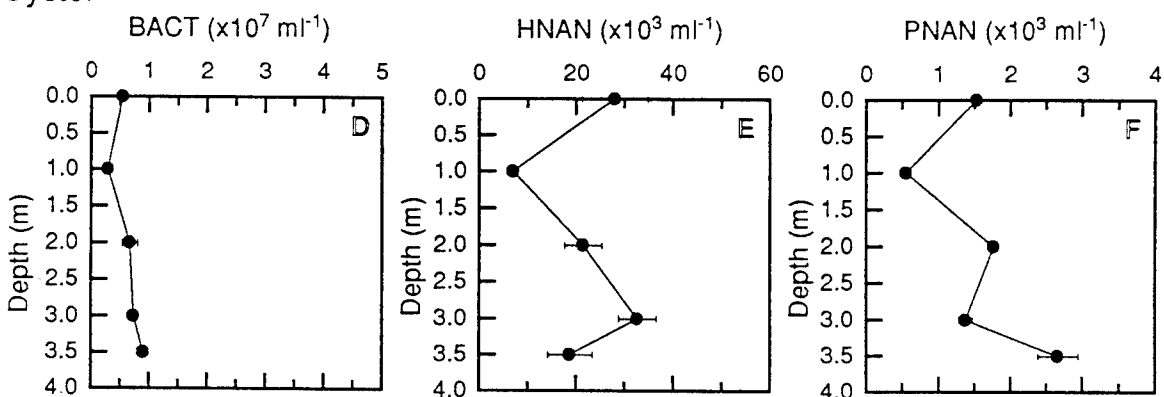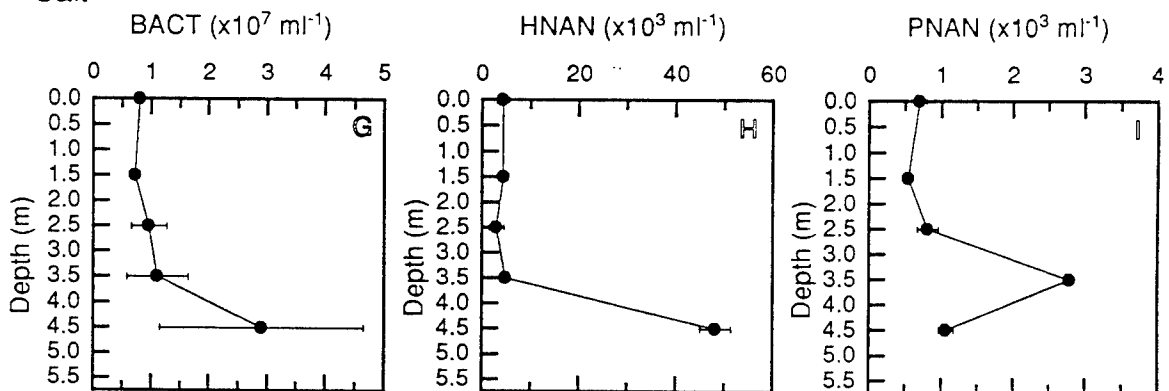
Figure 3.20. Vertical profiles of bacteria (BACT), heterotrophic nanoplankton (HNAN) and phototrophic nanoplankton (PNAN) abundances (±1 SD) in Perch Pond (A-C), Oyster Pond (D-F) and Salt Pond (G-I) determined during early summer 1993.

to ~3.3×10^4 HNAN ml^{-1}, but did not show any clear trend with depth (Fig. 3.20e). The depth distribution of heterotrophic nanoplankton in Salt Pond mirrored the depth distribution of bacteria in this pond (Fig. 3.20h). Heterotrophic nanoplankton abundances throughout the upper water column of Salt Pond were relatively constant (~5.2×10^3 HNAN ml^{-1}) and then increased nearly an order of magnitude in the thermocline to almost 5×10^4 HNAN ml^{-1} (Fig. 3.20h).

Phototrophic nanoplankton abundances in the three coastal ponds ranged from ~500 ml^{-1} to >3×10^3 ml^{-1} (Fig. 3.20c, f, i). Maximum PNAN abundances in Perch Pond occurred 0.5 m below the surface (~3×10^3 PNAN ml^{-1}). Below this depth PNAN abundances remained relatively constant (Fig. 3.20c). Phototrophic nanoplankton abundances fluctuated widely with depth in Oyster Pond and abundances ranged from 500 PNAN ml^{-1} to 2.7×10^3 PNAN ml^{-1} (Fig. 3.20f). PNAN abundances were relatively constant (<1×10^3 ml^{-1}) from surface waters down to the top of the halocline (~3m) in Salt Pond (Fig. 3.20i). A peak in the abundance of phototrophic nanoplankton (3×10^4 ml^{-1}) was observed near the middle of the halocline (3.5 m) in Salt Pond, and below this depth phototrophic nanoplankton abundances decreased to ~1×10^3 PNAN ml^{-1}.

The absolute and relative abundances of mixotrophic nanoplankton were low in all three coastal ponds (Fig. 3.21). Subsurface peaks in phototrophic nanoplankton abundance did not correlate with peaks in mixotrophic nanoplankton abundance in any of the ponds. The highest abundances of mixotrophic nanoplankton were observed in surface waters of Oyster Pond (~142 ml^{-1}, ~2% of total PNAN; Fig. 3.21b). Mixotrophic nanoplankton abundances in Perch Pond were highest at a depth of 1 m (~100 ml^{-1}), but comprised only 1.1% of the total PNAN assemblage (Fig. 3.21a). Mixotrophic nanoplankton were present in Salt Pond at less than 15 cells ml^{-1} and contributed less than 0.4% to the phototroph assemblage (Fig. 3.21c).

Microplankton were also enumerated in Salt Pond after significant numbers of mixotrophic ciliates (chloroplast-retaining ciliates) were observed. Ciliates dominated

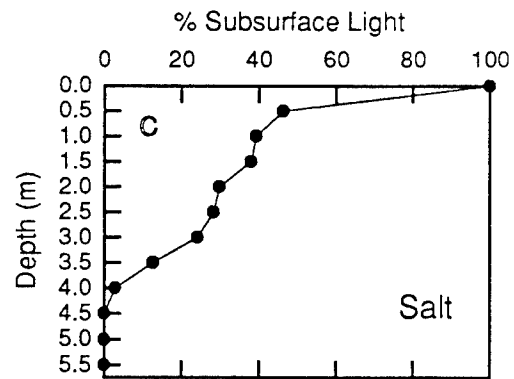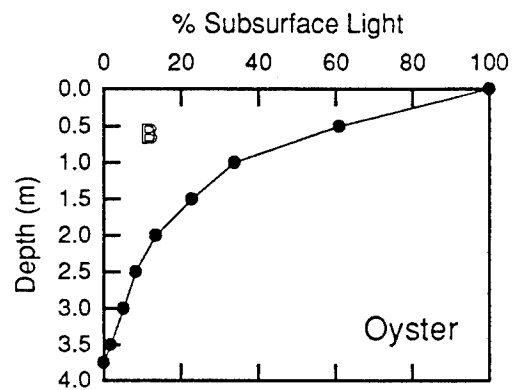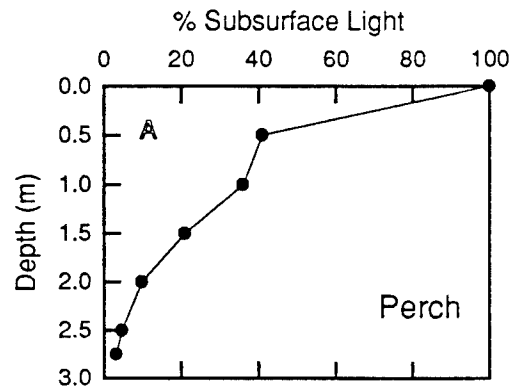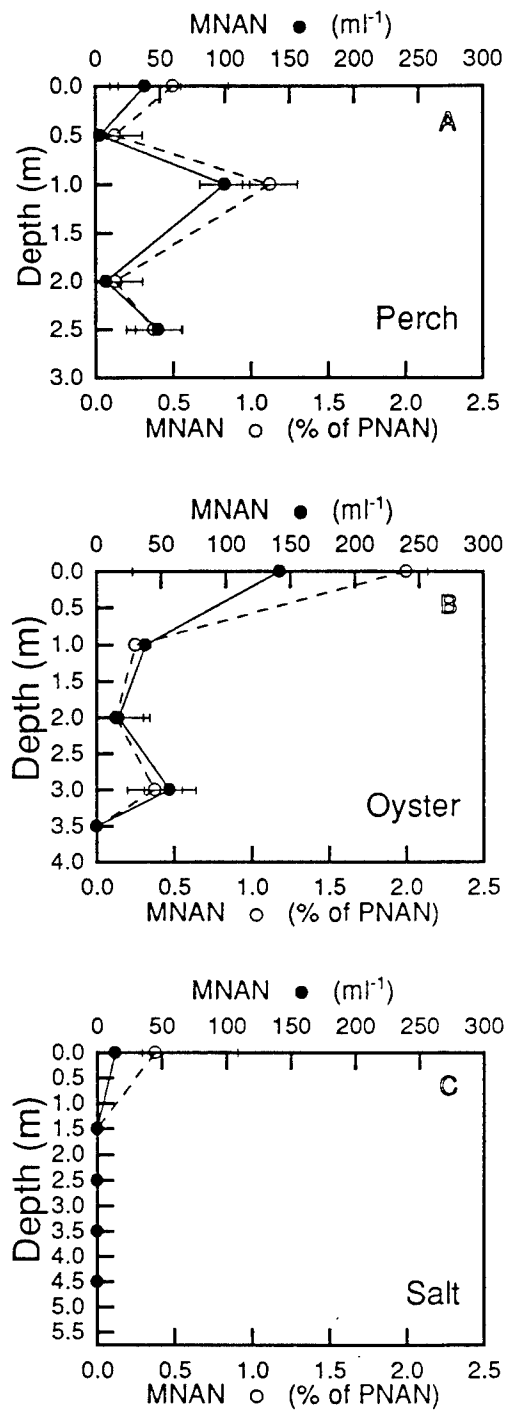Figure 3.21. Vertical profiles of mixotrophic nanoplankton absolute (●) and relative (○) abundance in Perch Pond (A), Oyster Pond (B) and Salt Pond (C), Falmouth, MA determined during early summer 1993.

the microplankton assemblage throughout the upper water column, and their total
abundances (heterotrophic and mixotrophic ciliates) were highest near the middle of the
halocline (3.5 m; >5x10^5 L^{-1}; Fig. 3.22). Other microplankton present at the time of
sampling included ebridians and dinoflagellates (data not shown). Ebridians were only
observed at

a depth of 3.5 m and did not exceed 1.2x10^3 L^{-1}. Dinoflagellates were present at all
depths, but were only numerically important in the halocline with abundances of ~2x10^5
L^{-1}. "Green" ciliates were observed throughout the upper water column of Salt Pond
(Fig. 3.22). Absolute abundances of these chloroplast-retaining ciliates were highest at a
depth of 3.5 m (1.4x10^5 L^{-1}), although the relative abundance of green ciliates was
highest at the surface (39% of total ciliate abundance) and decreased with depth (<27% at
3.5 m).
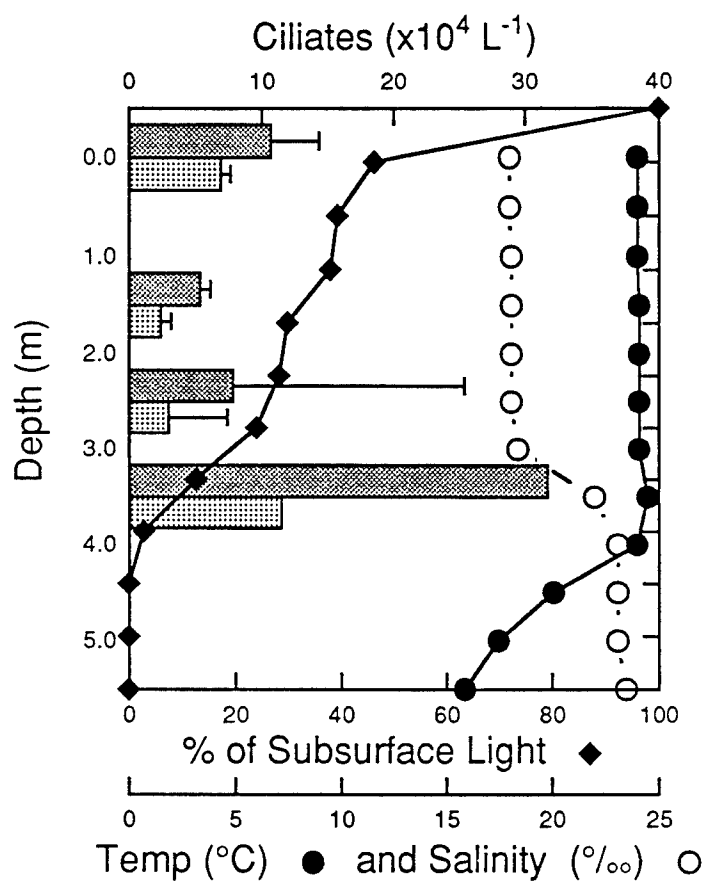
Figure 3.22. Vertical distribution and abundance (±1 SD) of heterotrophic ciliates
(▨) and mixotrophic ciliates (▨), and vertical profiles of temperature
(●), salinity (○) and light attenuation (◆) in Salt Pond, Falmouth, MA
on 17 July 1993.

DISCUSSION

Mixotrophic algae in Adirondack seepage ponds

Mixotrophic algae were a numerically important and abundant component of the total nanoplanktonic algal assemblage in only one pond examined during the seepage pond survey. Mixotrophic algae accounted for approximately 25% of the nanoplanktonic algal assemblage in the epilimnion and metalimnion of Wheeler Pond. Total abundances of mixotrophic algae were high in the metalimnion, and a large population of the mixotrophic chrysophyte *Dinobryon* was present at this depth ($1.7x10^4$ ml^{-1}). *Dinobryon* was less important in the epilimnion, and ~50% of these two upper water column populations of *Dinobryon* were phagotrophically active at the time of this study. Wheeler Pond was of moderate DOC concentration relative to the ponds surveyed. Mixotrophic algae in the upper water columns of two ponds of high DOC concentration (Amphitheater and Little Echo) were moderately important (15%), but absolute abundances were more than an order of magnitude lower (~$1x10^3$ ml^{-1}) than MNAN abundances in Wheeler Pond. The dominant forms of mixotrophic algae in the epilimnia of Amphitheater Pond and Little Echo Pond were unidentified solitary forms, unlike Wheeler Pond in which *Dinobryon* dominated the mixotroph assemblage. Mixotrophic nanoplankton abundances in the remaining seven ponds that were surveyed were very modest (<3% of the total nanoplanktonic algal assemblage), and the dominant forms of mixotrophic algae varied. If it is assumed that only 50% of the mixotroph populations in these other ponds were phagotrophically active, similar to the *Dinobryon* population in Wheeler Pond, the abundance of mixotrophic nanoplankton in these other ponds would still be modest.

One hypothesis for explaining mixotroph abundances in these ponds was predicated on the belief that some mixotrophic algae consume bacteria in order to obtain major nutrients (N, P). Ponds constituting a gradient in DOC concentration were

examined based on the assumption that DOC might stimulate bacterial production (Tranvik, 1988), and if the C:N:P ratio of the carbon substrate was high, increased bacterial production along the DOC gradient would increase competition for dissolved inorganic nutrients between bacteria and algae. The relative and/or absolute abundances of mixotrophic algae were therefore hypothesized to increase with increasing DOC concentration because mixotrophic algae may gain a selective advantage over other algae under conditions of increased nutrient stress by consuming bacteria directly.

While the ability to look for correlations in the data was constrained by the limited number of ponds and depths within ponds that could be sampled during the study period, clearly no relationship existed between the relative or absolute abundance of mixotrophic nanoplankton and pond DOC concentration. One reason may be that competition for nutrients between bacteria and phytoplankton did not increase along the DOC gradient, and therefore a selective advantage for mixotrophic algae may not have existed in high DOC ponds relative to low DOC ponds. It may have been that dissolved organic carbon compounds in these ponds were relatively inert and did not serve as substrate for bacterial production. Alternatively, DOC in the ponds may have been susceptible to bacterial degradation, but the carbon:nutrient ratio of the organic substrates may have been low. Bacterial uptake of dissolved inorganic nutrients therefore would not have been expected to increase with increasing DOC concentration. Dissolved inorganic nutrient concentrations in this study of nine ponds were, in fact, positively correlated with DOC concentration. This positive correlation is contrary to the hypothesis above. Thus, the carbon:nutrient ratio of the DOC and/or the relative stability of the DOC in these small forested ponds may have contributed to the lack of any clear relationship between mixotroph abundance and DOC concentration.

The mixotrophic chrysophyte *Dinobryon* was found to be numerically important in the upper water column of Wheeler Pond, and a peak in the abundance of *Dinobryon* occurred near the mid-metalimnion. It has been shown in laboratory experiments with individual species of mixotrophic algae, that these algae may benefit from phagotrophy in a number of different ways, including the acquisition of carbon, major nutrients or essential growth factors. Investigators have observed metalimnetic peaks of *Dinobryon* in several lakes and have suggested that phagotrophic activity was important in sustaining these populations at the low light levels experienced at these depths (Bird & Kalff, 1986; Bird & Kalff, 1987; Bird & Kalff, 1989). In laboratory studies with *D. cylindricum*, Caron *et al.* (1992) found that phagotrophic activity persisted in populations of *D. cylindricum* under low light conditions or short photoperiod. No net bacterial carbon incorporation occurred under these conditions, although population densities of *D. cylindricum* were maintained. Based on these results, Caron *et al.* (1992) suggested that phagotrophy may be an important carbon source for maintenance metabolism in suboptimal light regimes and may allow for the survival of this alga under these conditions. Light levels in the mid-metalimnion of Wheeler Pond at the time of this study were approximately 3% of subsurface light. The depth distribution of *Dinobryon* in this study and the large proportion of these cells that were phagotrophically active are therefore consistent with findings from other field and laboratory studies.

Interestingly though, phagotrophic activity by the *Dinobryon* population in the epilimnion was observed to be at a similar level (~50%) as in the metalimnetic population. It has been suggested that bacterivory is necessary for the growth of *Dinobryon* even in optimal light regimes, although phagotrophy may also allow for the survival of this alga in low light (Caron *et al.*, 1992). In experiments with *D. cylindricum* conducted in a variety of light regimes, it was found that this alga could not be

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maintained axenically in optimal light, and that *D. cylindricum* also could not survive in continuous darkness when provided with high concentrations of bacteria (Caron *et al.*, 1992). It was therefore hypothesized that this alga is an obligate photophagotroph, and that the primary function of phagotrophy may be to provide *Dinobryon* with major nutrients (N or P) or an essential organic compound(s) required for phototrophic growth (Caron *et al.*, 1992). Phosphate acquisition was suggested by Veen (1991) as the most likely advantage of bacterivory for *Dinobryon*, since *Dinobryon* generally occurs in oligotrophic environments (Sandgren, 1988) and bacteria are typically enriched in P (Vadstein *et al.*, 1988). Dissolved inorganic phosphate concentrations were less than 0.25 μM PO_4^{-3} in the epilimnia and metalimnia of Wheeler Pond during this study, and this PO_4^{-3} concentration has been shown to limit the growth of two species of *Dinobryon* in culture experiments (Lehman, 1976). Although the phagotrophic activity of *Dinobryon* in Wheeler Pond is consistent with the hypothesis that this behavior is important as a source of P for this alga, the results are not conclusive, and the possibility remains that the primary function of phagotrophy in *Dinobryon* may be to provide specific growth factors required for phototrophic growth.

Mixotrophy in coastal salt ponds

This is the first investigation of the occurrence of mixotrophic algae in small coastal lagoons. The abundances of mixotrophic algae in the nanoplankton size range were low (<150 ml^{-1}) and they were numerically unimportant (<2% of total nanoplanktonic algal abundances) throughout the water column in all three coastal salt ponds at the time of the study. Even if only 50% of the mixotrophic algae present were phagotrophically active and absolute abundances of mixotrophic nanoplankton were doubled, mixotrophic algae would still have comprised an insignificant fraction of the total nanoplanktonic algal assemblage in these ponds. Therefore, even though the sample

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size was small (only three sites examined), the consistently low abundances observed during this study suggest that mixotrophic nanoplankton may not be common in semi-enclosed coastal lagoons, at least during summer. This is in contrast to other coastal (Dahl et al., 1989; Johnsen & Lein, 1989; Børsheim et al., 1990), open ocean (see Chapter 2) and freshwater environments (Bird & Kalff, 1986; Kimura et al., 1986; Porter, 1988; Sanders & Porter, 1988; Bird & Kalff, 1989; Bennett et al., 1990; Sanders, 1991; Berninger et al., 1992), where mixotrophic algae can be a highly significant component of the nanoplankton assemblage. These small coastal lagoons are typified by the aperiodic formation and breakdown of sharp salinity gradients, however, due to their relatively shallow bathymetry and wind-driven circulation. In contrast to species inhabiting freshwater or the open ocean and other coastal waters, species inhabiting these semi-enclosed coastal ponds must be tolerant of changing salinity (euryhaline). An inability of species of mixotrophic nanoplankton to tolerate shifts in salinity would therefore account for their relative unimportance in these coastal salt ponds.

While mixotrophic nanoplankton were not common in these coastal ponds at the time of study, mixotrophic ciliates (chloroplast-retaining ciliates capable of photosynthesis and phagotrophy) were found to be an abundant component of the microplankton (20-200 µm) assemblage in one of the ponds. Several different species of "green" ciliates (chloroplast-retaining ciliates) were present at relatively high abundances throughout the upper water column of Salt Pond. Chloroplast-retaining ciliates appear to be common in estuarine, coastal and offshore areas (Blackbourn et al., 1973; Beers et al., 1980; Laval-Peuto & Febvre, 1986; Stoecker et al., 1987; Stoecker et al., 1989). During a previous study in adjacent nearshore waters conducted by Stoecker et al. (1987), mixotrophic ciliates were found to be a numerically important (42% of total ciliates) component of the total microplanktonic ciliate assemblage in surface waters in spring and summer. During this study, "green" ciliates also comprised a large fraction (39%) of the microplanktonic ciliate assemblage in surface waters. Interestingly though, abundances

of mixotrophic ciliates throughout the water column in Salt Pond were higher and exceeded abundances previously observed in surface waters of the adjacent nearshore areas (Stoecker *et al.*, 1987). The impact of larger grazers of microplanktonic ciliates was not examined and may have been important in the development of high abundances of mixotrophic ciliates at this site.

The relative importance of mixotrophic ciliates decreased with depth in Salt Pond as the abundance of heterotrophic ciliates increased. Absolute abundances of both mixotrophic ($\sim 1.4 \times 10^5$ L^{-1}) and heterotrophic ciliates ($>3.6 \times 10^5$ L^{-1}) were greatest at a depth of 3.5 m which coincided with the mid-halocline. The halocline was reinforced by a rapid decrease in temperature suggesting that a very strong pycnocline existed in the region of the ciliate maximum. High abundances of total ciliates observed at a depth of 3.5 m in Salt Pond therefore may have been a consequence of the unique physical feature present at this depth.

Although mixotrophic ciliates were documented in Salt Pond at high abundances, they were not observed in the other two coastal ponds examined in this study. Other microplanktonic mixotrophs were, however, observed in one of the other ponds at low concentrations. One or two microplanktonic dinoflagellates containing chlorophyll and ingested fluorescent tracers were observed in two samples from Perch Pond. Recently, mixotrophic dinoflagellates that were feeding primarily on nanociliates were also documented in regions of the Chesapeake Bay estuary (Bockstahler & Coats, 1993a; Bockstahler & Coats, 1993b). It appears that while mixotrophy may not be prevalent among the nanoplankton assemblage in these small coastal lagoons during summer, this trophic behavior may be important for some microplanktonic species in these environments. The influence of mixotrophy in the transfer of energy and nutrients in estuarine microbial food webs may therefore differ markedly from that in freshwater and open ocean plankton communities where nanoplanktonic mixotrophs can be more common.

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CHAPTER 4

The Importance of Phagotrophy in the Nutrition of Mixotrophic Nanoplankton in an Adirondack Seepage Pond

ABSTRACT

The importance of phagotrophy for the growth and survival of mixotrophic algae was investigated in a meso-oligotrophic seepage pond located in the west-central region of the Adirondack Mountains, NY,. A six day *in situ* experiment was conducted in enclosures to examine the effect of changes in bacterial abundance (with addition of labile organic carbon), inorganic nutrient supply and light intensity on algal phagotrophy. *Dinobryon* and other unidentified solitary and colonial algae exhibited phagotrophic behavior during the experiment. *Dinobryon* comprised a large fraction of the phototroph assemblage at the end of the experiment in treatments with higher bacterial abundances and in low light. Experimental manipulations did not appear to significantly affect the relative abundance of the unidentified solitary mixotrophs. Unidentified colonial mixotrophs appeared to be favored at *in situ* light levels with increased bacterial abundances.

The phagotrophic activity of *Dinobryon* was examined separately. Activity (% of cells found to be ingesting fluorescently labeled bacteria) was low in the presence of elevated concentrations of dissolved inorganic N and P at *in situ* light levels, but high in controls and glucose amended enclosures without added N and P. In low light, phagotrophic activity was also high. These results are in good agreement with previous hypotheses (Veen, 1991; Caron *et al.*, 1992) that have suggested the primarily role of phagotrophy in *Dinobryon* may be to provide specific growth factors or major nutrients, and that under light limiting conditions this trophic behavior may also allow for the survival of this alga. Observations indicated that phagotrophic activity in the *Dinobryon* population persisted at a low level even in the presence of favorable phototrophic conditions (*i.e.* *in situ* light with added N and P), but was higher when N and P were not added. These results may indicate that consumption of a few bacteria $cell^{-1}$ d^{-1} may be important for continued good growth of *Dinobryon* regardless of light intensity or

nutrient availability, but that during periods of low nutrient availability, phagotrophic uptake of bacteria may serve as an important source of N or P. It was therefore hypothesized that nutrient acquisition is probably an important secondary function of phagotrophy in *Dinobryon* that may influence grazing activity, but that the primary function of phagotrophic behavior may be to provide essential growth factor(s).

INTRODUCTION

Mixotrophic algae are unicellular algae that combine autotrophic and heterotrophic modes of nutrition, commonly phototrophy and phagotrophy. Algal species in more than 25 genera (Chysophyta, Pyrrhophyta and Cryptophyta) have been reported to exhibit phagotrophy, and pigmented members of other flagellate taxa containing morphologically similar chlorotic and apochlorotic species may also be capable of phagotrophy (Sanders & Porter, 1988, for summary of references). Phagotrophy therefore apparently is widespread among phytoflagellates, yet its potential importance in algal growth and survival has been examined in only a few species. Based on these studies, several hypotheses have been put forward to explain the role of phagotrophy in algal nutrition. These include acquisition of carbon, major nutrients (N or P) or specific growth factors (*e.g.* vitamins, phospholipids).

Phagotrophy is the primary source of carbon used for metabolism and growth in some mixotrophic algae. Photosynthesis in these algae appears to be important as a survival strategy under poor heterotrophic conditions (Andersson *et al.*, 1989; Sanders *et al.*, 1990). For example, Sanders *et al.* (1990) found in studies with the mixotrophic chrysophyte *Poterioochromonas malhamensis* that this primarily heterotrophic species was able to increase chlorophyll production and survive phototrophically when bacterial prey densities fell below a grazing threshold of approximately 1×10^6 ml^{-1}. At bacterial concentrations above the grazing threshold, however, photosynthesis did not contribute more than 7% to the alga's total carbon budget. Similar trophic behavior has also been observed in some species of *Ochromonas* (Fenchel, 1982; Andersson *et al.*, 1989).

Other species of mixotrophic algae are obligate photophagotrophs that require both photosynthesis and phagotrophy for their growth and survival. Phagotrophy has been hypothesized to provide these algae with a source of organic compounds that they are otherwise unable to synthesize but which they require for phototrophic growth.

Uroglena americana is a freshwater red tide chrysophyte that appears to exhibit this trophic strategy. Kimura and Ishida (1986) reported that light and ingestion of bacteria were both necessary for growth of this alga. Based on laboratory fractionation studies, they determined that bacterivory by *U. americana* was associated with acquisition of an essential phospholipid required for growth (Kimura & Ishida, 1989).

Phagotrophy might also provide mixotrophic algae with major nutrients (N & P). This trophic strategy has been hypothesized for mixotrophic algae in oligotrophic environments (Veen, 1991). Support for this hypothesis has been provided by a study of several species of mixotrophic algae present during two toxic algal blooms that occurred off the coast of Norway (Nygaard & Tobiesen, 1993). The authors found that low orthophosphate concentration, a feature of the blooms, resulted in higher bacterial uptake rates in a number of the algae tested. Further evidence is provided in Chapter 2. Higher abundances of mixotrophic algae occurred in oligotrophic surface waters at several stations in the Sargasso Sea. Based on the results of experimental manipulations conducted with surface water samples, it was suggested that mixotrophic algae may supplement major nutrients via phagotrophy, and therefore hypothesized that this behavior may allow these algae to survive in competition with other phototrophs during periods of nutrient limitation (Chapter 2). Other investigators have also suggested that supplementation of major nutrients (N or P) may be one reason for phagotrophy in *Dinobryon*, a freshwater Chrysophyte commonly found in oligotrophic lake environments (Veen, 1991; Caron *et al.*, 1992).

Until recently, the literature contained only anecdotal accounts of algal phagotrophs in nature. Little attention was directed towards the potential role of mixotrophic algae in energy and nutrient flow through planktonic food webs. With recent advancements in microbial methodology though (Porter & Feig, 1980; Caron, 1983; Sherr & Sherr, 1983; Sherr *et al.*, 1987; Kemp *et al.*, 1993), and recognition of the importance of microheterotrophic processes in planktonic food webs (Pomeroy, 1974; Azam *et al.*,

1983), there has been a renewed interest in algal phagotrophy, and ecologists have begun to establish the importance of these algae in aquatic systems. Several studies have indicated that phagotrophic algae may dominate the phototroph assemblages of some plankton communities (Bird & Kalff, 1986; Bird & Kalff, 1989; Berninger et al., 1992, also Chapters 2 & 3). In addition to dominating photosynthetic processes on occasion, mixotrophic algae may also contribute significantly to total bacterivory. For example, in several freshwater lake environments, algal bacterial consumption was found to exceed consumption by the heterotrophs (Bird & Kalff, 1986). Four species of the Chrysophyte *Dinobryon*, constituting a large biomass in Lac Cromwell, Quebec, were found to be major consumers of bacteria. In Quinn Bay, Lac Memphremagog, Vermont-Quebec, they removed more bacteria from the water column than the crustaceans, rotifers and ciliates combined. In the surface waters of Lake Oglethorpe, Georgia, grazing by the autotrophic community constituted 55% of total bacterial grazing of all microflagellates, ciliates, rotifers and crustaceans combined (Porter, 1988; Sanders et al., 1989). While these studies demonstrate that algal phagotrophy can at times account for a substantial portion of the total grazing pressure on bacterioplankton, the role of algal phagotrophy in the flow of energy and materials through planktonic food webs is poorly understood.

A survey of nine Adirondack seepage ponds was conducted to provide information on the abundance and distribution of mixotrophic algae (Chapter 3). During this study, mixotrophic algae, most notably *Dinobryon*, were found to be important contributors (>25%) to the phototrophic nanoplankton assemblage in the epilimnion and metalimnion of a small (area, 6 ha; Z_{max}, 18 m), meso-oligotrophic (total P, ~30 μg L^{-1}; total N, ~180 μg L^{-1}; DOC, ~5.7 mg L^{-1}, see Chapter 3) pond (Wheeler Pond) located in the west-central region of the Adirondack Mountains near Old Forge, NY. The results from a field manipulation experiment designed to provide information on the importance of light, nutrients and prey density in determining the abundance and phagotrophic

activity of the mixotrophic nanoplankton population in the epilimnion of this pond is now presented.

METHODS

The experiment was conducted *in situ* with whole water samples that were
subdivided, treated and incubated in enclosures for a period of 6 days. Abundances of the
microbial populations, including mixotrophic nanoplankton, were monitored for changes
with respect to additions of inorganic nutrients and/or labile organic carbon, and reduced
light intensity. Water was collected from a depth of 1 m using a 4 L Van Dorn bottle and
pooled in 20 L carboys. Subsamples were immediately removed and preserved to a final
concentration of 1% gluteraldehyde in order to establish the initial population densities of
heterotrophic picoplankton (bacteria <2 μm in size) and phototrophic and heterotrophic
nanoplankton (algae and protozoa 2-20 μm in size). All population counts were
performed by epifluorescence microscopy at 1000x magnification (Porter & Feig, 1980;
Sherr *et al.*, 1993).

Water was removed from the carboys in 3.5 L aliquots, placed in duplicate 4 L
clear polycarbonate enclosures and treated as described below.

C	Control, water with no additions
G	Water with additions of glucose (2.5 mg L^{-1} final conc.) every other day
NP	Water with additions of N-NH$_4^+$ (20 μM final conc.) and P-PO$_4^{-3}$ (2 μM final conc.) every other day
GNP	Water with additions of glucose, N and P every other day at above concentrations
LL	Water at low light intensity (<5% incident light) without inorganic or organic additions
LLGNP	Water at low light intensity with additions of glucose, N and P every other day at above concentrations

It was hypothesized that several specific responses would be accomplished by the
manipulations. Glucose was added to stimulate bacterial growth. In the treatment with
added glucose only, stimulation of the bacterial assemblage would be expected to
increase their uptake of dissolved nutrients thereby resulting in increased competition for

nutrients with the phototroph assemblage. Reduced dissolved nutrient concentrations may potentially favor mixotrophic algae that are able to acquire nutrients by consumption of bacteria. The purpose of combining additions of glucose with additions of inorganic N and P was to increase bacterial abundances without creating nutrient limiting conditions for the phototrophs. Mixotrophic species in enclosures with these additions may benefit from the higher prey abundances. N and P were added alone to stimulate the growth of phototrophs without stimulating bacteria. Only primarily heterotrophic species of mixotrophs and obligate photophagotrophs would presumably continue to consume bacteria in enclosures treated in this way. Finally, light intensity was reduced to decrease photosynthetic rate, and nutrients and glucose were added to stimulate the heterotrophic nanoplankton and bacteria in these enclosures. Algae capable of supplementing photosynthetic carbon by consuming bacterial C may be favored in the low light treatment without added C, N or P, while primarily heterotrophic algae would presumably be favored in the presence of higher bacterial abundances and low light expected in the LLGNP treatment.

On day six, population abundances of bacteria and heterotrophic and phototrophic nanoplankton were determined again in each enclosure as described earlier to examine the response of these populations to each experimental manipulation. The following procedure also was carried out at the beginning and end of the six-day incubation experiment to determine the responses of the mixotrophic nanoplankton population to experimental manipulations. Mixotrophic nanoplankton abundances were determined by removing 200 ml subsamples from each experimental enclosure, placing them in 250 ml clear polycarbonate vessels and incubating them *in situ* with fluorescently labeled prey for a period of five hours to trace ingestion by algae. Mixotrophic algae are defined operationally by this method as cells that contain both autofluorescent chloroplasts and ingested fluorescently labeled prey. Fluorescently labeled bacteria (FLB) prepared from *Pseudomonas halodurans* according to the method

of Sherr *et al.* (Sherr *et al.*, 1987) were used as tracers of ingestion in this experiment. FLB were added at a final concentration equal to 1×10^6 FLB ml^{-1}. Immediately after the addition of fluorescently labeled prey the containers were gently swirled to distribute the tracer, and samples were removed and preserved to establish the initial numbers of labeled prey. Following the incubations, samples were preserved with NiCl$_2$ (0.05% to prevent egestion; Waterbury, pers. comm.) followed by gluteraldehyde (1%) and examined by epifluorescence microscopy at 1000x magnification to establish the percent of phototrophic cells that were phagotrophic.

The percent of phototrophic cells that were phagotrophic was determined by observing more than 200 individual phototrophic nanoplankton (chloroplast-bearing cells 2-20 μm in size) for ingested FLB as they were encountered in each T$_{final}$ FLB incubated sample during the microscopical examinations. The method is based on two assumptions: (1) fluorescently labeled prey are ingested at the same rate as natural prey, and (2) all mixotrophic algae actively phagocytize prey during experiments. Thus, in cases where a specific mixotrophic alga is recognizable under epifluorescence, the percent of these cells with ingested FLB provides a measure of phagotrophic activity for the alga. In an algal assemblage of unknown composition though, the percent of cells with ingested FLB may be used to provide a lower limit estimate of the relative abundance of mixotrophic algae, but may not accurately reflect the phagotrophic activity of the mixotroph population, since both phototrophic and mixotrophic algae may comprise the assemblage.

Comparisons of day 0 mean abundances and % of phagotrophically active algae with day 6 mean values were performed (T-test, $P<0.05$). Statistical contrasts were then conducted using the results from day 6, and treatments were contrasted to controls using transformed data (ln or -1/x). Significant differences were determined at a significance level of $P<0.05$.

RESULTS

Abundances of bacteria and heterotrophic and phototrophic nanoplankton in controls were not found to be significantly different ($P<0.05$) between day 0 and day 6 (Table 4.1). Heterotrophic bacterial abundance remained stable in controls over the course of the six day incubation period (2.4-2.8 $\times 10^6$ ml^{-1}; Table 4.1). Although significant differences were not observed, the mean heterotrophic nanoplankton abundance in controls increased >4 fold and the mean total phototrophic nanoplankton abundance decreased ~1.5 fold in controls during the experiment. Within the phototrophic nanoplankton assemblage, it was possible to identify the mixotrophic chrysophyte *Dinobryon* as well as other phototrophic algae including *Dactylococcopsis*, *Gleocystis*, cryptophytes and dinoflagellates. Other phototrophic nanoplankton could not be identified using epifluorescence microscopy and thus, were grouped according to whether they existed as solitary or colonial forms. Mean abundances of *Dinobryon* decreased more than an order of magnitude in controls during the experiment (Table 4.1). Mean abundances of *Dactylococcopsis*, cryptophytes, dinoflagellates and unidentified solitary PNAN also decreased in controls during the experiment. Differences between day 0 and day 6 values for *Dinobryon* and these other algae were not statistically significant, though, due to large variances and the patchy nature of some colonial algae. Mean abundances of *Gleocystis* and unidentified colonial PNAN in controls did not exhibit large changes in their abundances over the course of the six day incubation period (Table 4.1).

Of the algae that could be identified using epifluorescence microscopy, only the known mixotroph *Dinobryon* was observed with ingested FLB. Unidentified solitary and colonial individuals also exhibited phagotrophic activity. The percent of *Dinobryon* cells that were phagotrophically active remained constant in controls over the course of the experiment (Table 4.2). The percent of unidentified solitary and colonial PNAN that

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Table 4.1. Population abundances (±1 SD) in controls determined at the beginning and end of a 6 day experimental incubation performed in Wheeler Pond, Adirondack Mtns., NY from 7-13 August, 1992. Based on a T-test of the means (P-value=0.05), abundances on Day 6 were not significantly different from abundances on Day 0.

Cell Type	Day 0	Day 6
Bacteria ($\times 10^6$ ml^{-1})	2.82±0.34	2.38±0.20
Heterotrophic Nanoplankton ($\times 10^4$ ml^{-1})	1.21±0.44	5.44±0.10
Phototrophic Nanoplankton ($\times 10^4$ ml^{-1})	3.56±2.31	1.90±0.90
Dinobryon ($\times 10^2$ ml^{-1})	219.60±220.95	17.08±19.63
Dactylococcopsis ($\times 10^2$ ml^{-1})	6.86±6.92	1.07±1.51
Gleocystis ($\times 10^2$ ml^{-1})	29.97±11.42	41.10±2.26
Cryptophytes ($\times 10^2$ ml^{-1})	1.25±1.55	0.00±0.00
Dinoflagellates ($\times 10^2$ ml^{-1})	0.40±0.80	0.00±0.00
Unidentified Solitary PNAN ($\times 10^2$ ml^{-1})	67.61±36.10	22.42±7.55
Unidentified Colonial PNAN ($\times 10^2$ ml^{-1})	27.13±40.12	21.35±13.59

Table 4.2. Percent (±1 SD) of phototrophic nanoplankton that were phagotrophically active in controls determined at the beginning and end of a 6 day experimental incubation performed in Wheeler Pond, Adirondack Mtns., NY from 7-13 August, 1992. Significant differences (P-value=0.05) between Day 0 and Day 6 values were not detectable.

Cell Type	Day 0	Day 6
% of *Dinobryon*	49.35±25.17	49.00±10.75
% of Unidentified Solitary PNAN	19.68±9.64	3.95±2.19
% of Unidentified Colonial PNAN	28.61±34.52	0.00±0.00

were mixotrophic decreased in controls during this experiment, although significant differences again were not detected.

Bacterial abundances (BACT) in enclosures that received additions of nitrogen and phosphorus (NP) or glucose, N and P (GNP, LLGNP) increased more than 3 fold relative to controls (Fig. 4.1a). Mean bacterial abundances for these treatments ranged from 7.4×10^6 BACT ml^{-1} to 8.4×10^6 BACT ml^{-1}. Smaller increases in the abundances of bacteria were observed in the glucose treated enclosures (G, increase of <65%) and the low light enclosures without added carbon or nutrients (LL, increase of <30%). Abundances in untreated enclosures (C) were $\sim 2.4 \times 10^6$ BACT ml^{-1}. Assuming that a similar grazing pressure on bacterioplankton existed in all enclosures, these results indicate that bacterial production may have been limited by N or P, or co-limited by carbon substrate and N or P, rather than by carbon substrate alone.

Heterotrophic nanoplankton (HNAN) responded similarly to the bacteria during the study (Fig. 4.1b). HNAN abundances increased between 2.4 fold and 5.4 fold relative to controls (5.4×10^3 HNAN ml^{-1}) in all five treatments, presumably due to the increase in bacterial prey abundance that occurred in the treated enclosures. Maximum abundances were observed in the NP, GNP and LLGNP treatments with values between 2.4×10^4 and 2.9×10^4 HNAN ml^{-1}.

The total PNAN abundance in treatments ranged from $\sim 1.2 \times 10^4$ ml^{-1} to $>4.1 \times 10^4$ ml^{-1} (Fig. 4.1c). No significant differences were detectable between total PNAN abundances in treatments and controls at the end of the experiment. Mean total PNAN abundances in low light treatments were ~30% (LL) and ~10% (LLGNP) lower than controls. Their abundances in enclosures with added glucose only and with added inorganic nutrients only were ~20% (G) and ~40% (NP) higher than in controls. In enclosures that received additions of glucose together with inorganic nitrogen and phosphorus, total PNAN abundances were approximate ~2 fold higher than in controls.
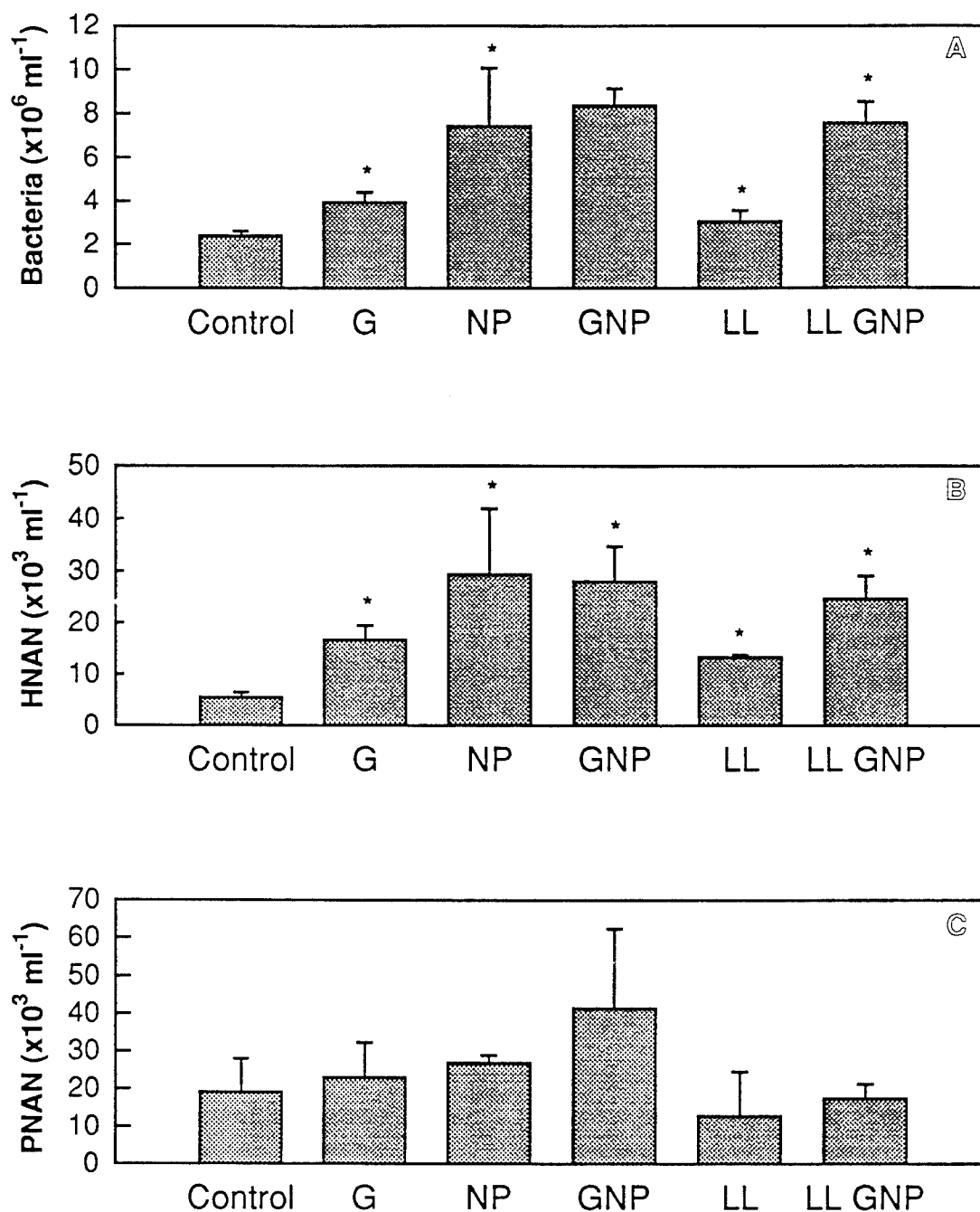
Figure 4.1. Mean population abundances (±1 SD) of bacteria (A), heterotrophic nanoplankton (B), and phototrophic nanoplankton (C) in controls, glucose (G), nitrogen+phosphorus (NP), glucose+nitrogen+phosphorus (GNP), low light (LL) and light with glucose+nitrogen+phosphorus (LLGNP) treatments at the end of a 6 day experimental incubation conducted from 7-13 August 1992 in Wheeler Pond, Adirondack Mtns., NY. Phototrophic nanoplankton includes solitary and colonial forms with cells sizes <20 μm in diameter. Significant differences between treatments and controls are indicated by "*" (P-value = 0.05). See text for details of treatments.

The combined abundance of *Dactylococcopsis*, *Gleocystis*, cryptophytes and dinoflagellates (algae not found to ingest prey during this study) fell to less than 20% ($<1x10^3$ ml^{-1}) of control abundances in enclosures receiving combined additions of glucose, nitrogen and phosphorus (GNP, LLGNP; Fig. 4.2a). In the untreated low light enclosures, controls, and enclosures with added glucose (G) or added N and P only (NP), abundances were similar to abundances in controls ($\sim4x10^3$ ml^{-1}). Algal groups that contained phagotrophically active algae responded differently from non-phagotrophic algae (Fig. 4.2b-d). Mean abundances of *Dinobryon* were 4.3 to 4.8 times higher than controls in treatments incubated at ambient light intensity and provided with additions of inorganic nutrients and/or glucose (G, NP, GNP). Smaller increases in *Dinobryon* abundances (~2.5 fold) occurred in response to low light (LL, LLGNP; Fig. 4.2b). Due to the highly patchy nature of *Dinobryon* (colonial genus) though, these differences were not statistically significant.

The unidentified solitary PNAN responded similarly to *Dinobryon* (Fig. 4.2d). Abundances increased 3.3 to 5.3 fold in the G, NP and GNP treatments, while abundances of unidentified solitary PNAN were similar to controls in the low light treatments (LL, LLGNP). In contrast, unidentified colonial PNAN abundances increased significantly in the treatment with added glucose and inorganic nutrients incubated in ambient light (6.5 fold in GNP; Fig. 4.2e). In enclosures with added glucose only (G) or added inorganic N and P only (NP), abundances of unidentified colonial PNAN changed only slightly relative to controls (G, ~66% increase; NP, ~30% decrease). Almost no unidentified colonial PNAN survived in the two low light treatments.

Dinobryon was phagotrophically active in all the treatments. A large percentage of *Dinobryon* cells (40-50%) were actively ingesting bacteria in the controls (C), the treatment with added glucose (G), and in both low light treatments (LL, LLGNP). Phagotrophic activity by *Dinobryon* was much lower (<10%) in ambient light treatments enriched with dissolved N and P (NP & GNP; Fig. 4.3a). Unidentified solitary algal

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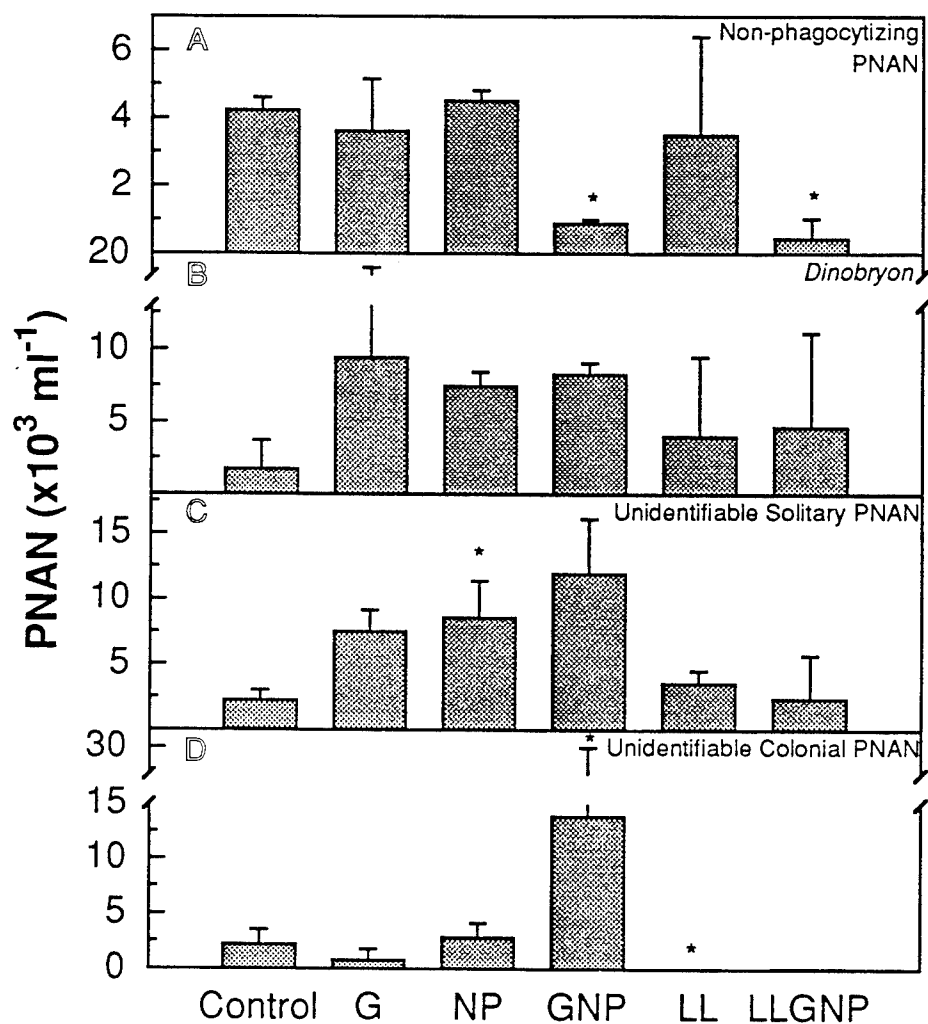
Figure 4.2. Combined abundance (±1 SD) of nanoplanktonic phototrophs in groups that were not phagotrophic (A), total abundance (±1 SD) of *Dinobryon* (B), total abundance of unidentified solitary phototrophic nanoplankton (C) and total abundance of unidentified colonial nanoplankton (D) in controls, glucose (G), nitrogen+ phosphorus (NP), glucose+nitrogen+phosphorus (GNP), low light (LL) and low light with glucose+nitrogen+phosphorus (LLGNP) at the end of a 6 day experimental incubation conducted from 7-13 August 1992 in Wheeler Pond, Adirondack Mtns., NY. Significant differences between treatments and controls are indicated by "*" (P-value = 0.05). See text for details of treatments.

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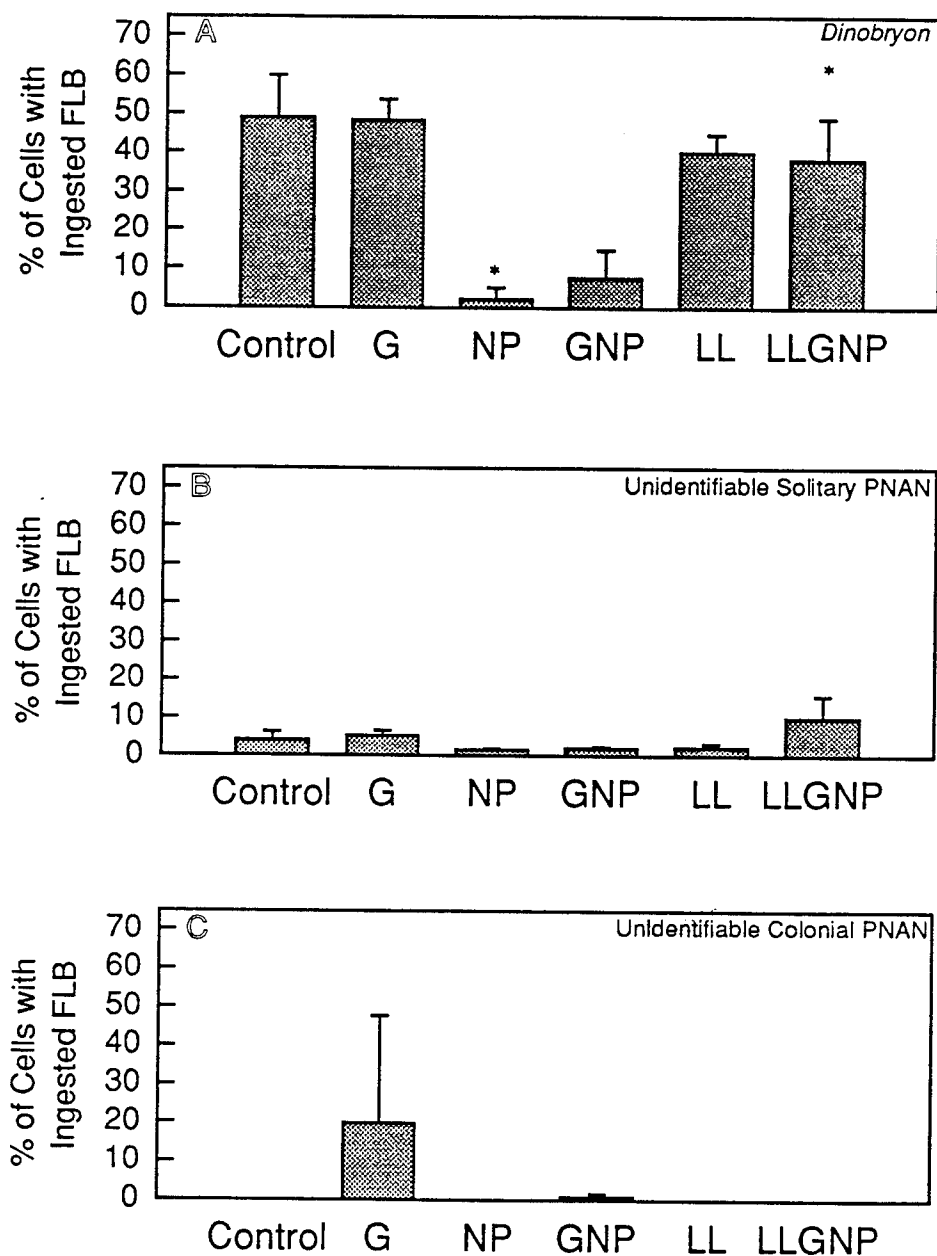
Figure 4.3. Percent (±1 SD) of *Dinobryon* cells that were phagotrophically active (A), and percent (±1 SD) of solitary (B) and colonial (C) phototrophic nanoplankton that were mixotrophic in controls, glucose (G), nitrogen+phosphorus (NP), glucose+ nitrogen+phosphorus (GNP), low light (LL) and low light with glucose+nitrogen+ phosphorus (LLGNP) treatments at the end of a 6 day experimental incubation conducted from 7-13 August 1992 in Wheeler Pond, Adirondack Mtns, NY. Significant differences between treatments and controls are indicated by "*" (P-value = 0.05). See text for details of treatments.

phagotrophs were observed in all five treatments and in controls, similar to *Dinobryon*. However, a very small percentage (generally <5%) of the total unidentified solitary PNAN assemblage was mixotrophic in each treatment (Fig. 4.3b). Likewise, little or no mixotrophs were detectable among the unidentified colonial PNAN assemblage in most of the treatments (Fig. 4.3c). In the treatment with added glucose, however, approximately 20% of the unidentified colonial PNAN appeared to be phagotrophic.

A persistent problem with enclosure experiments is that a limited number of treatments are possible and the investigator must often choose between increasing the number of treatments or increasing the number of replicates. The data from this experiment suffered from a high degree of variability due primarily to too few replicates, but also due to difficulties in counting as a consequence of large amounts of particulate material in the samples and the patchy distribution of some colonial algae. Transformations of the data did not prove helpful in homogenizing the variances.

DISCUSSION

Importance of mixotrophic unidentified solitary and colonial algae

The dominant forms of nanoplanktonic algae in an earlier study of the phytoplankton composition in the epilimnion of Wheeler Pond included *Synura*, *Gleocystis*, *Chlamydomonas*, *Dactylococcopsis*, *Chromulina* and *Dinobryon* (Saunders, 1992). *Gleocystis*, *Dactylococcopsis* and *Dinobryon* were also identified during this study. Of the solitary forms identified by Saunders, *Chromulina* has been observed to ingest particles (Hutner & Provasoli, 1951; Bird & Kalff, 1987). This alga may have comprised some portion of the unidentified solitary mixotroph population during this study. Ingestion by unidentified colonial phototrophs was also observed in the enclosures. While *Synura* is not known to ingest particles (D.A. Caron, WHOI, pers. comm.), *Uroglena* and *Chrysosphaerella* are capable of phagotrophy (Sanders & Porter, 1988). *Uroglena*-like and *Chrysosphaerella*-like colonies were observed during this experiment and these morphotypes were found to be ingesting prey. It is therefore possible that some portion of the unidentified colonial mixotroph assemblage was comprised of these mixotrophic species at the time of this study.

The unidentified solitary PNAN assemblage in each of the treatments was comprised of relatively few mixotrophs (<5%) at the end of the experiment. There abundances at the beginning of the experiment were apparently higher, though, and it may be that negative effects from containment masked the potential effects of bacterial abundance, nutrient concentration and/or light in determining the relative abundance of unidentified solitary mixotrophs in this pond. Unidentified colonial mixotrophs did not persist in controls and containment may have had a large negative effect on their survival. They did, however, persist in two treatments (G & GNP), and comprised ~20% of the unidentified colonial assemblage in these two treatments. It is important to note that

while all of the *Dinobryon* cells present during the study were presumably capable of phagotrophy, no more than 50% of the individuals of this mixotrophic chrysophyte genus were ever found to be phagotrophically active in any of the treatments. Assuming that the unidentified mixotrophic colonial algae in this pond also had a similar distribution of cells with ingested FLB, the relative abundance of unidentified colonial mixotrophs could have been twice as large in the G and GNP treatments than was reported, and they may also have been present at very low abundances in the other treatments. Nearly 40% of the unidentified colonial PNAN could therefore have been mixotrophic in the glucose amended treatment. It is therefore possible that additions of glucose in the absence of added nutrients significantly influenced the relative abundance of unidentified colonial mixotrophs. Higher abundances of bacteria and a possible reduction in the availability of N or P in the glucose amended treatment may have favored some colonial mixotrophic forms.

Importance of phagotrophy in the nutrition of Dinobryon

The mixotrophic chrysophyte *Dinobryon* comprised a large fraction of the phototroph assemblage during this study. This alga is typically a member of the phytoplankton assemblage in dimictic lakes and ponds characterized by circum-neutral pH and low nutrient loading. During periods of stratified water, *Dinobryon* frequently dominates the nanoplankton assemblage in these systems (Sandgren, 1988). Phagotrophic behavior was first described in this alga in 1911 by Pascher. Ecologists did not begin to focus studies on the role of phagotrophy in the nutrition of *Dinobryon*, or the influence of environmental factors on the trophic behavior of this alga, until much later however. Such studies were initiated only after Bird and Kalff (1986; 1989) demonstrated that *Dinobryon* could have an important impact on grazing and that this

alga may supplement up to 50% of its photosynthetic carbon by consuming bacteria under low light conditions.

Several hypotheses have since been proposed to explain the importance of phagotrophy in the growth and survival of *Dinobryon*. Based on laboratory studies with *D. divergens*, Veen (1991) hypothesized the following: (1) that *Dinobryon* is facultatively phagotrophic and does not have an absolute need for bacteria; (2) that phagotrophic activity in *Dinobryon* is dependent on the type of limiting condition (*e.g.* elevated phagotrophic activity with light limitation vs. reduced phagotrophic activity with iron limitation) and not on the intensity of the limitation; and (3) that the main advantage of phagotrophic behavior in *Dinobryon* must be nutrient acquisition, not carbon acquisition. Veen concluded that to experience an advantage from carbon acquired from bacteria, *Dinobryon* must grow under carbon- and light-limited conditions. Yet, *Dinobryon* normally occurs in rather clear, well-lighted oligotrophic phosphorus-deficient waters. Based on the studies with *D. divergens*, Veen suggested that light may influence phagotrophic behavior, but reasoned that the most likely benefit from bacteria must be phosphorus because the C:P ratio of bacteria was considerably less than that of phytoplankton (Vadstein *et al.*, 1988) and the occurrence of *Dinobryon* was typically associated with low phosphorus conditions.

Caron *et al.* (1992) also examined the importance of bacterivorous behavior in laboratory studies with axenic and bacterized cultures of *D. cylindricum*. Based on their experiments, they concluded that *D. cylindricum* is an obligate phototroph, but also requires bacteria for sustained vigorous growth. They further hypothesize based on their results, that the primary function of phagotrophic behavior in this species may be to provide essential growth factor(s) or major nutrients for phototrophic growth, but that this behavior may also allow for the survival of individuals when light is limiting.

The results of this study provide the first, although preliminary, field evidence in support of hypotheses that have suggested that the primary function of phagotrophy in

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Dinobryon is as a source of specific growth factors or major nutrients for photosynthesis, and that this behavior may also allow for the survival of this alga in low light. *Dinobryon* was found to be a relatively important contributor to the phototroph assemblage in low light treatments, and phagotrophy appeared to play an important role in their nutrition under these conditions. In both low light treatments, regardless of the presence or absence of added nutrients, phagotrophic activity was high. Little or no increase in population abundance was apparent, though. These results are in good agreement with the results from the study by Caron *et al.* (1992). They found that in short photoperiod or very low light intensity, *D. cylindricum* consumed bacteria and was able to maintain its population densities, but did not increase in population density. Under these conditions the net efficiency of incorporation for C, N and P into algal biomass was zero.

In addition, high abundances of *Dinobryon* were observed in treatments that were incubated in ambient light with added glucose and/or major nutrients. Under conditions that presumably should have favored purely phototrophic species (ambient light and added major nutrients), *Dinobryon* was an important member of the phototroph assemblage, and only exhibited a low level of phagotrophic activity. *Dinobryon* was also an important contributor to the phototroph assemblage in the treatment with added glucose, but phagotrophic activity was much higher than in the nutrient enriched treatments. Phagotrophic activity was also high in controls, although the phototroph assemblage was dominated by non-phagocytizing algae. These results indicate that phagotrophic activity in *Dinobryon* growing under high light conditions may be most advantageous when these cells are nutrient limited.

During the survey of Wheeler Pond two weeks prior to this study it was found that dissolved inorganic PO_4^{-3} was less than 0.46 μM (Chapter 3). These concentrations have been found to limit the growth of two species of *Dinobryon* in culture experiments (Lehman, 1976). Higher levels of phagotrophic activity observed in the controls and the glucose treatment relative to nutrient supplemented treatments indicate that phagotrophy

may be important as a source of N or P for *Dinobryon*. The small fraction of phagotrophically active *Dinobryon* cells observed in NP and GNP treatments indicated that consumption continued at a low level even in the presence of added nutrients, though. A few bacteria cell^{-1} d^{-1} may therefore be important for good growth of this alga in the light.

The preliminary results of this study are consistent with the hypothesis that nutrient acquisition is a secondary function for phagotrophy in *Dinobryon* that may influence grazing activity by this alga, and that the primary function of phagotrophic behavior in *Dinobryon* may be to provide essential growth factors. The nutrient status of aquatic environments where *Dinobryon* is abundant may therefore play a role in determining the grazing impact of this alga and its contribution to energy and nutrient flow in planktonic food webs.

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CHAPTER 5

Summary and Perspectives

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SUMMARY AND PERSPECTIVES

A large part of this dissertation has been devoted to expanding our knowledge of the occurrence of mixotrophs in aquatic ecosystems, primarily unicellular mixotrophic algae in the nanoplankton size range (2-20 μm), in order to provide a basis for incorporation of mixotrophy into microbial food web trophodynamics. Much of the work conducted so far on this topic has targeted freshwater environments. This dissertation, however, contains the first detailed reports on the vertical distribution and abundance of mixotrophic nanoplankton in an oligotrophic open ocean environment and in semi-enclosed coastal lagoons. In addition, further documentation is provided on the occurrence of mixotrophic nanoplankton is some newly examined freshwater plankton communities. Specifically, the absolute and relative abundances of mixotrophic nanoplankton were documented at nine stations in the Sargasso Sea (Chapter 2), in three coastal salt ponds located in Falmouth, Massachusetts, (Chapter 3), and in nine freshwater seepage ponds located in the Adirondack Mountains of New York (Chapter 3).

In summary, mixotrophic algal abundances (MNAN) in surface waters of the Sargasso Sea in spring ranged from less than a few MNAN ml^{-1} to approximately 140 MNAN ml^{-1}. These abundances represented from <5% to more than 50% of the nanoplanktonic algal assemblage. Despite the fact that absolute abundances of mixotrophic algae in the coastal ponds during summer were similar to absolute abundances in surface waters of the Sargasso Sea (<15 MNAN ml^{-1} to 142 MNAN ml^{-1}), mixotrophic algae were not a major component of the total nanoplanktonic algal assemblage (<2%) in any of these coastal ponds. Mixotrophic algae were found to be an important component of the nanoplanktonic algal assemblage in at least one of the Adirondack seepage ponds during summer. Absolute abundances of individuals of the mixotrophic chrysophyte genus *Dinobryon* ranged from ~2.5x10^3 to ~2x10^4 ml^{-1} in the

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upper water column of Wheeler Pond and nearly 50% of this population was phagotrophically active. Phagotrophically active *Dinobryon* cells along with other solitary and colonial mixotrophic algae accounted for nearly 25% (1.1×10^4 ml^{-1}) of the total nanoplanktonic algal assemblage in this pond. Mixotrophic algae in two other seepage ponds accounted for ~15% of the nanoplanktonic algal assemblage with abundances $<1 \times 10^3$ ml^{-1}. Mixotrophic algal abundances ranged from <200 ml^{-1} to ~1×10^3 ml^{-1} in the remaining six ponds that were examined, but their numerical contribution to the total nanoplanktonic algal assemblage was less than 5% in each of the ponds. The results presented in this dissertation and in other studies (Bennett *et al.*, 1990; Berninger *et al.*, 1992) demonstrate that the occurrence of mixotrophic nanoplankton is a widespread phenomenon in aquatic ecosystems, but clearly their abundances and numerical importance vary (Table 5.1).

Phagotrophy has been hypothesized to play a variety of roles in the nutrition of mixotrophic algae, which include acquisition of carbon, major nutrients (N and P) or specific growth factors (*i.e.* vitamins, phospholipids) important for the growth and survival of these algae. Factors that affect the phagotrophic behavior of these algae presumably are important in determining the distribution and abundance of mixotrophic algae in nature and may influence their contribution to bacterivory. The remainder of this dissertation addresses the importance of certain physical, chemical and biological factors in determining the abundance of mixotrophic algae in nature. Chapters 2 and 4 report the results of field experiments that were designed to examine the influence of light, dissolved nutrients and prey density on mixotrophic nanoplankton abundances in surface waters of the Sargasso Sea and in one Adirondack seepage pond . There was evidence from these experiments that the abundance of mixotrophic algae and/or their phagotrophic activity decreased with nutrient enrichment. The results from experiments in the Sargasso Sea provided support for the hypothesis that phagotrophy allows mixotrophic algae in oligotrophic surface waters of this open ocean environment to

Table 5.1. Abundances of mixotrophic nanoplankton in marine and freshwater plankton communities. Mixotrophs were identified as individual cells 2-20 μm in size with autofluorescent chloroplasts and ingested fluorescently labeled tracer particles using epifluorescence microscopy. "+" indicates mixotrophic nanoplankton abundances were <5% of total nanoplanktonic algal abundances. In cases where no relative abundance is reported, known mixotrophic species were enumerated, but % mixotrophy was not documented.

Site	Species	Abundance (ml⁻¹)	% of Total Algal Nanoplankton	Reference
Open Ocean				
31°51.90N, 64°49.80W	algal nanoplankton	<26	+	Chapter 2
32°07.05N, 64°19.30W	algal nanoplankton	<19	+	Chapter 2
31°51.90N, 64°19.30W	algal nanoplankton	<6	+	Chapter 2
31°53.58N, 63°58.80W	algal nanoplankton	<6	+	Chapter 2
32°07.58N, 64°20.20W	algal nanoplankton	<4	+	Chapter 2
32°06.80N, 64°19.91W	algal nanoplankton	144	53	Chapter 2
32°05.00N, 64°21.21W	algal nanoplankton	69	50	Chapter 2
25°59N, 64°30W	algal nanoplankton	25	6	Chapter 2
30°26N, 64°30W	algal nanoplankton	38	+	Chapter 2
Coastal				
Perch Pond, MA	algal nanoplankton	100	+	Chapter 3
Oyster Pond, MA	algal nanoplankton	148	+	Chapter 3
Salt Pond, MA	algal nanoplankton	15	+	Chapter 3
Freshwater				
Round Pond, NY	algal nanoplankton	186	+	Chapter 3

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Freshwater (continued)

Location	organism			Reference
Deer Pond, NY	algal nanoplankton	456	+	Chapter 3
Pitchfork Pond, NY	algal nanoplankton	1.1×10^3	+	Chapter 3
Wheeler Pond, NY	algal nanoplankton	1.1×10^4	25	Chapter 3
Kanacto Pond, NY	algal nanoplankton	370	+	Chapter 3
Rat Pond, NY	algal nanoplankton	368	+	Chapter 3
Amphitheater Pond, NY	algal nanoplankton	792	16	Chapter 3
Fox Pond, NY	algal nanoplankton	144	+	Chapter 3
Little Echo Pond, NY	algal nanoplankton	878	14	Chapter 3
Lake Giles, PA	algal nanoplankton	22	+	Beminger et al., 1992
Lake Lacawac, PA	algal nanoplankton	$440 - 1.1 \times 10^3$	22 - 48	Beminger et al., 1992
Lake Waynewood, PA	algal nanoplankton	19	+	Beminger et al., 1992
Lake Oglethorpe, GA				
February	algal nanoplankton	$<200 - 1 \times 10^3$	14	Bennett et al., 1990
April	algal nanoplankton	380 - 560	16	Bennett et al., 1990
May	algal nanoplankton	~20	+	Bennett et al., 1990
June	algal nanoplankton	~10 - 20	6	Bennett et al., 1990
September	algal nanoplankton	20 - 210	11	Bennett et al., 1990
Lac Memphremagog, Quebec	*Dinobryon*	479		Bird & Kalff, 1986
Lac Gilbert, Quebec				
June 1984	*Dinobryon*	$<2 \times 10^3 - \sim 1 \times 10^4$		Bird & Kalff, 1987
July 1986	*Dinobryon*	$<300 - >1.5 \times 10^3$		Bird & Kalff, 1989
Lac Bowker, Quebec	phytoplankton	30		Bird & Kalff, 1989

compete with other purely phototrophic algae during periods of low nutrient concentrations. This trophic behavior may therefore allow mixotrophic algae to become numerically importance in surface waters of the Sargasso Sea on occasion. The efficiency of nutrient cycling in open ocean microbial food webs may therefore be enhanced during periods of low nutrient concentrations as a consequence of nanoplanktonic algal phagotrophy. Further studies that address the contribution of photosynthesis and phagotrophy to the growth of mixotrophic nanoplankton and their contribution to bacterivory in open ocean environments may substantiate this hypothesis.

Preliminary results obtained from the experiment conducted in Wheeler Pond suggested that the availability of nutrients affected the phagotrophic activity of members of at least one mixotrophic chrysophyte genus (*Dinobryon*) common in temperate and northern oligotrophic lakes. The percent of *Dinobryon* individuals that were phagotrophically active was found to decrease with nutrient enrichment, but the actual abundances of this mixotrophic alga appeared to increase under these conditions. Phagotrophy by *Dinobryon* persisted to some degree even with nutrient enrichment, though. The results from this experiment, although preliminary, therefore supported the hypothesis that phagotrophy by *Dinobryon* may be primarily important as a means of acquiring specific growth factors, but suggested that nutrient availability may influence the level of phagotrophic activity in this alga, and may therefore play a role in determining the importance of this alga to total bacterivory. *Dinobryon* has been found to have an important impact on total bacterivory in some freshwater plankton communities (Bird & Kalff, 1986; Sanders *et al.*, 1989). Future research that focuses on the role of specific nutrients and other factors (*i.e.* the nutritional quality of bacterial prey) in controlling the rate of phagotrophic activity in *Dinobryon* may therefore help determine when and where this mixotroph will have a large impact on bacterivory and the flow of energy and nutrients through microbial food webs.

Experiments to examine factors that may influence the abundance of mixotrophic nanoplankton in the coastal ponds were not conducted. Although the relative importance of mixotrophic nanoplankton was found to be consistently low in these ponds at the time of study, mixotrophic ciliates in the microplankton size range (20-200 μm) were found to be relatively abundant in one coastal salt pond. Mixotrophic dinoflagellates were also observed in another pond during the study. A few studies have recently documented the presence of high abundances of mixotrophic dinoflagellates and ciliates in the microplankton assemblage of some estuarine environments (Stoecker *et al.*, 1981; McManus & Fuhrman, 1986; Stoecker *et al.*, 1987; Stoecker *et al.*, 1989; Bockstahler & Coats, 1993). Although mixotrophy can be common among the nanoplankton assemblage in freshwater, open ocean and other coastal environments (Bird & Kalff, 1986; Bird & Kalff, 1987; Bird & Kalff, 1989; Dahl *et al.*, 1989; Johnsen & Lein, 1989, Chapter 2; Bennett *et al.*, 1990; Berninger *et al.*, 1992), this trophic behavior may be more prevalent among the microplankton assemblage in estuarine environments.

Energy and nutrient cycling in aquatic environments in which mixotrophy predominates in the microplankton assemblage is likely to differ from energy and nutrient cycling in environments in which this trophic behavior is absent or predominates in the nanoplankton assemblage. Most of the regeneration of inorganic nutrients in aquatic microbial food webs is commonly thought to occur at the level of the nanoplankton (Fig. 5.2, Food Chain I). Mixotrophic nanoplankton that consume bacteria to obtain nutrients presumably are more efficient at retaining these nutrients than their heterotrophic counterparts. Nutrients may therefore be transferred more efficiently from bacteria to higher trophic levels in some freshwater and open ocean plankton communities where mixotrophic nanoplankton may predominate, particularly during periods of nutrient limitation. Most of the nutrients regenerated under these conditions would more than likely occur at the level of the microplankton (Fig. 5.2, Food Chain II). Nutrient transfer in plankton communities where mixotrophic microplankton

predominate (*e.g.* some estuarine environments) would presumably be enhanced as well, but the level of enhancement may not be as great as in the previous scenario because a large fraction of the nutrients would be regenerated by heterotrophic nanoplankton prior to reaching the microplankton assemblage (Fig. 5.2, Food Chain III).

Microbial Food Chain I	Microbial Food Chain II	Microbial Food Chain III
6.4g C 0.9g N **copepods** C:N = 7 GGE = 10% ↑	28g C 4g N **copepods** C:N = 7 GGE = 10% ↑	16g C 2.3g N **copepods** C:N = 7 GGE = 10% ↑
64g C 9.1g N **heterotrophic ciliates** C:N = 7 GGE = 40% ↑	280g C 40g N **heterotrophic ciliates** C:N = 7 GGE = 40% ↑	160g C 22.8g N **mixotrophic ciliates** C:N = 7 GGE (N) = 100% ↑
160g C 22.8g N **heterotrophic flagellates** C:N = 7 GGE = 40% ↑	700g C 100g N **mixotrophic flagellates** C:N = 7 GGE (N) = 100% ↑	160g C 22.8g N **heterotrophic flagellates** C:N = 7 GGE = 40% ↑
400g C 100g N **bacteria** C:N = 4	400g C 100g N **bacteria** C:N = 4	400g C 100g N **bacteria** C:N = 4

Figure 5.2. Hypothetical transfer of energy (C) and nutrients (*e.g.* nitrogen) through microbial food chains dominated by heterotrophic nanoplankton and microplankton (I), mixotrophic (obligate photophagotrophy) nanoplankton and heterotrophic microplankton (II), and heterotrophic nanoplankton and mixotrophic (chloroplast-retaining) microplankton (III). Heterotroph gross growth efficiencies (GGE) and C:N ratios taken from Caron (1991).

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BIOGRAPHICAL NOTE

Andrea L. Arenovski graduated from Dennis-Yarmouth Regional High School (MA) in June 1984 and entered the University of North Carolina at Wilmington (UNCW) in August 1984. Andrea later graduated *summa cum laude* from UNCW in May 1988 with a Bachelor of Science degree in Biology and Marine Biology (double concentration). She entered the MIT/WHOI Joint Program in Biological Oceanography immediately following graduation from UNCW. Her first three years of graduate school were spent studying the physiological ecology of salt marsh cordgrass (*Spartina alterniflora*) in the Great Sippewissett Salt Marsh under the direction of Brian L. Howes. Andrea later joined the laboratory of David A. Caron in June of 1991. Her work on the ecology of mixotrophic algae is described in this thesis which was completed in September 1994. Andrea has accepted a position with the Massachusetts Bay Marine Studies Consortium in Boston, MA, and begins work as Program Manager and Director of Science in November 1994.

Honors and Awards

- WHOI Summer Student Fellowship, 1987
- Who's Who in American Universities and Colleges, 1987
- UNCW Undergraduate Research Fellowship, 1988
- National Collegiate Natural Sciences Award, 1988
- Switzer Environmental Fellowship, 1989
- Ocean Ventures Fund Award, 1992

Professional Societies

- Sigma Xi Scientific Research Society
- American Society of Limnology and Oceanography
- Estuarine Research Federation
- North East Estuarine Research Society

Publications

- Arenovski, A.L. & B.L. Howes. (1992). Lacunal allocation and gas transport capacity in the salt marsh cordgrass *Spartina alterniflora. Oecologia* 90: 316-322.

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- Arenovski, A.L. (in prep) The importance of phagotrophy in the nutrition of mixotrophic nanoplankton in an Adirondack seepage pond.

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16. Abstract (Limit: 200 words)
Mixotrophic algae are algae that combine photosynthesis with phagotrophy to satisfy nutritional requirements. The vertical distribution and abundance of mixotrophic algae 2-20 μm in size was documented in the Sargasso Sea, and in nine freshwater seepage ponds (Adirondack Mountains, NY) and three coastal salt ponds (Falmouth, MA). Mixotrophic nanoplankton abundances in the Sargasso Sea ranged from less than a few cells ml^{-1} to more than 140 cells ml^{-1}, and they were more abundant in surface waters. On two occasions mixotrophic algae comprised more than 50% of the total algal nanoplankton assemblage in surface waters. The results of field experiments indicate that phagotrophy may provide mixotrophic algae in surface waters of the Sargasso Sea with major nutrients and may enable them to compete with purely phototrophic algae during periods of low nutrient concentrations.

Mixotrophic nanoplankton abundances comprised a small fraction of the total algal nanoplankton (<5%) in most of the freshwater seepage ponds and in all three coastal salt ponds. Mixotrophic algae in the epilimnion of one freshwater pond, however, accounted for ~25% of the total algal nanoplankton. Results from field experiments that examined the phagotrophic behavior of the mixotrophic Chrysophyte *Dinobryon*, the dominant mixotroph in this pond, support the hypothesis that nutrient acquisition is an important secondary function for phagotrophy in *Dinobryon*, but that the primary function may be to provide essential growth factors.

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